Analytical challenges in the research of structure and functionality of humic substances from surface water

Dissertion for the degree of Doctor Scientarium
by
Tone Charlotte Gadmar

DEPARTMENT OF CHEMISTRY

FACULTY OF MATHEMATICS
AND NATURAL SCIENCES

UNIVERSITY OF OSLO
June 2005

Nos habebit humus

Acknowledgements

Science is a collective art. No scientific question is solved in isolation. A Dr.Scient. thesis like this one would not be possible without a large supporting network of colleagues, family and friends. The present work has been financed by and carried out at the University of Oslo in the Department of Chemistry. I am extremely grateful for this opportunity and my years here. Not only for getting a chance to evolve and mature as a scientist, but also for the teaching and administrative experience I have gained during my stay.

My first and most profound thanks go to my supervisors Rolf Vogt, Hans Martin Seip and Egil Gjessing for all your help, support and patience. Even though you may not all have been officially appointed as supervisors, you have certainly filled that function and each of you have taken care of different aspects of my development. Special thanks also go to the Cand.Scient. students that I in my turn got the chance to work with on different projects and partly tutor. Three of them; Bjørn Østerhus, Lars Evje and Magnus Christiansen have made contributions to the papers included in this thesis. Thanks guys! It was a pleasure to work together with you and I have learned a lot from this experience.

There are also a large number of scientific colleagues in Norway and abroad that deserve to be mentioned: Atle Hindar and Christian Vogelsang from The Norwegian Institute for Water Research for the collaboration on the Terrestrial Liming and Chitosan Coagulation projects. Monika Takács and Jim Alberts (University of Georgia, USA), Per Kristian Egeberg (Agder College, Norway) and Janusz Pempkowiak (Institute of Oceanology, Poland) are gratefully acknowledged for lending out data originating from the NOM typing project and for allowing these to be reused and analyzed in a new setting in the terrestrial liming study. Many other colleagues have contributed with valuable discussion, criticism, information and encouragement deserving of mention. I would use this opportunity to send out a great thanks to all of the participants of the NOMiNiC and NOM-typing projects and my colleagues in the Nordic Chapter of the International Humic Substances Society (IHSS). I cannot imagine a more cooperative, including and friendly scientific union. You all deserve great honor and gratitude for your ability to welcome and encourage young scientists.

Finally, my love and gratefulness goes to my family. My parents Marianne Gadmar and Kjell Mykkelbost, and my sister Gry Mykkelbost, for your never ending love and encouragement. To Øystein, my love and my best friend, my greatest discussion partner and toughest and maybe most honest critic. I could not have done this without you. Ten great years have we had together: A period of life in which we both have evolved in our respective areas of science, shared interest for each others work and a lot of other subjects, and in which we have built a family. To our wonderful kids; Nikoline, Sofie and the little one stomping his little feet in my belly as I write, for all the joy you bring. With all your “Why”, “What” and “How” and the curiosity of children you remind me every day what science is really about. Have you had time lately to look at a newly planted tree wondering how tall it would grow? And when did you last look a snail in the eyes and ask it where it is going?

Tone C. Gadmar

Oslo, June 2005

Abstract

The study of natural organic carbon structure and functionality faces great analytical challenges due both to the substantial temporal and spatial variations found in nature, and the complexity of the humic matter itself. The most significant features of humic substances are their size and the lack of a well-defined chemical structure. Any study of these compounds can at best provide information on the average and span of properties assigned to this complex mixture. This poses challenges on all analytical levels in humic research, from instrument performance to the ultimate interpretation of the structure and functionality of natural organic carbon. In this thesis, covering four research articles, different analytical challenges in the reseach of humic matter structure and functionality on various levels are presented:

- An intercalibration study covering the merits and possible artefacts of the high temperature combustion method for determination of organic carbon compounds, revealed that the merits of these methods when used on marine samples could not be directly adapted to cover samples of terrestrial/aquatic origin. Samples of high concentrations and with refractory materials proved to be the most challenging and were likely to be underestimated. A substantial overconfidence regarding the limit of detection and analytical performance was revealed, likely to be the result of using syntetic and more combustible standard materials for method calibration.

- In a critical challenge of the NOM XAD-8 fractionation technique, it was confirmed that this method is of a highly operational and chromatographic nature. The boundary between the hydrophobic and hydrophilic fractions was revealed to be weak and dependent on concentration. The hydrophilic fraction changed gradually to a more aromatic content during the fractionation, and refractionation of hydrophilic and hydrophobic fractions revealed the establishment of a new secondary cut-off between hydrophobic and hydrophilic matter.

- In a study on the use of Chitosan and Chitosan/iron as coagulants for the removal of natural organic matter in drinking water, the physio-chemical properties of the residual organic matter after the treatment were studied by XAD-8 fractionation, fluorescence and UV-VIS spectroscopy scans. It was demonstated by combining these methods that the treatment preferentially removed the larger, more aromatic and hydrophobic entities of the organic matter and that the combination of Chitosan and iron proved to be the most efficient treatment with synergistic effects between the coagulants. The XAD-8 fractionation also revealed that the residual organic matter regained some of its previous hydrophobic character under storage. This effect was assigned to a secondary aggregation effect of organic matter commonly observed in fractionation and filtration studies.

- In a study covering the effect of liming on natural organic matter structure and mobility, data from two large research projects – the Gjerstad liming experiment and the NOM-typing project – were combined with great success. Through the combination of these data sets, increased charge density as a result of an increase in pH was seen to be the major mechanism behind the increased mobilization of organic matter observed in the stream. This increased mobilisation due to higher pH was also

found to trigger the release of older, more aromatic organic carbon containing acids of higher pK_a values otherwise concidered to be less mobile at lower pH.

List of papers

Paper I: *The merits of the high temperature combustion method for determining the amount of natural organic carbon in surface freshwater samples.*
Tone Charlotte Gadmar, Rolf David Vogt and Bjørn Østerhus.
International Journal of Environmental Analytical Chemistry. 2002, Vol. 82(7), p. 451-461.

Paper II: *Artefacts in XAD-8 NOM fractionation.*
Tone Charlotte Gadmar, Rolf David Vogt and Lars Evje.
International Journal of Environmental Analytical Chemistry. 2005, Vol. 85(6), p 365-376.

Paper III: *The nature of the residual organic matter in drinking water following treatment with Chitosan and combined Chitosan / iron coagulants.*
Tone Charlotte Gadmar and Magnus Christiansen
Aqua. Submitted.

Paper IV: *The effect of forest liming on humic mobilisation and physicochemical properties.*
Tone Charlotte Gadmar, Atle Hindar and Egil Gjessing
Science of the Total Environment. Submitted.

Abbreviations

DIC – Dissolved Inorganic Carbon
DOM – Dissolved Natural Organic Carbon
DOC – Dissolved Organic Carbon
HiX – Humification Index
HPI – Hydrophilic Fraction
HPOA – Hydrophobic Acids
HPON – Hydrophobic Neutrals
Kow – Octanol-water partitioning coefficient
LOD – Limit of Detection
NMR – Nuclear Magnetic Resonance
NOM – Natural Organic Carbon
NPOC – Non-Purgeable Organic Carbon
OC – Organic Carbon
POM – Particulate Organic Matter
RO – Reverse Osmosis
SAR – Specific Absorption Ratio
SOP – Standard Operational Procedure
sUVa – Specific UV Absorption
TOC – Total Organic Carbon

Table of Contents

1. Introduction

1.1 General introduction

What is so interesting and intriguing about decaying matter? Until about half a century ago humic substances were a neglected in most environmental studies with the exception of few and scattered publications by individual scientists reporting on their presence and quality (Aschan 1908, Oden 1919). Even though peat and peat land was valued as important natural resources, the humic substances were simply regarded as the product of natural decay, to refractory and random to hold any major environmental functions. Or maybe the scientific task was just too complex to embark on and the considered benefit of such research was considered to low to justify the needed investment. But as the general awareness of environmental issues grew during the sixties and seventies, so did the interest in humic matter research. During the eighties and nineties the interest has exploded into a large number of individual research fields as more and more key features and functions of humic matter has been revealed. Humic substances, or natural organic material (NOM) as it often is also named, have been found to be present in nearly all terrestrial, aquatic and marine environments and is today known to be involved in a large number of geochemical processes such as weathering, pH buffering, metal complexation and transport of pollutants, as well as being a significant biological source of carbon, nutrient and energy (Thurman 1985, Aiken et al. 1985, Jones 1992, Tranvik 1992, Steinberg 2003).

Research on humic matter is challenging because of the very nature of the substance. As stated above humic substances are considered to be the result of the natural decay of organic matter followed by a chemical or biochemical induced condensation into larger organic entities thermodynamically more stable and inert then the original substrate. The resulting substances will therefore have a longer lifetime in the environment than simpler biochemical molecules. The input material into the natural generation of humic substances may vary with the availability in different environments. Humic substances are known to be produced in situ in the terrestrial environment, as well as in aquatic and marine environment, but partly through different chemical pathways: In the terrestrial environment humic substances are generally considered to be mainly the product of lignin and cellulose degradation by chemical and microbial enzymatic processes followed by a chemical condensation and polymerization into larger entities (Thurman 1985, Aiken et al. 1985, van Loon and Duffy 2002), while in the aquatic and marine environments humic substances are considered to be mainly the product of Maillard condensation with sugars and amino acids as substrate (Aiken et al. 1985, Steinberg 2003). In the marine environment it is also suggested mechanisms of humic substance formation based on radical auto oxidative cross linkage with lipids as substrate (Harvey et al. 1983). This difference in substrate and synthetic pathways leaves clear differences in the structures of humic substances originating from terrestrial, aquatic and marine environments, but maybe more intriguing is they also have amazing similarities and hold many of the same key functions of their respective environments.

Some of the most significant features of humic substances are their size and the lack of defined and ordered chemical structure. In any given environment or any sample the humic matter will always be represented as a complex mixture of acidic and polyfunctional entities with a huge variation in size, composition and variation between aliphatic and

aromatic structure. In the literature their molecular size is described to span from a few hundred to several hundred thousand Daltons (Stumm & Morgan 1981, Aiken et al. 1985, Thurman 1985), although most sources would regard 500-5000 Dalton to be the common size terrestrial- and approximately 500-2000 Dalton for aquatic humic matter (Aiken et al. 1985, Thurman 1985). The size of marine humic matter is rarely found to exceed 1200 Dalton (Tait 1981, Aiken et al. 1985). As the composition of humic matter is about 50% carbon (in weight), a span from 500-5000 Dalton would correspond to roughly 20 to 200 carbon atoms per individual entity. Even though structures of a size below this size also are included in the term NOM, most researchers would hesitate to regard these as humic substances since they would have a much clearer resemblance with the original substrate and to a large degree can be characterized as individual chemical species by their proper names. Above the size of 5000 Dalton it can be argued where the boundary between molecular size and the size of more loosely associated aggregates should be drawn and literature do not agree unanimously on this point, probably because this is a very difficult point to investigate and determine and would be highly dependent on the present matrix of the surroundings. The acidity of humic matter originates mainly from a mixture of carboxylic- and phenolic acids in a polyfunctional mixture randomly distributed among the individual humic molecular entities. A small fraction of functional groups produce positively charged sites that hold important potential for the complexation of negatively charged substances. Depending on origin, age and size of the humic molecules it may be more or less aromatic versus aliphatic in structure, and given the total variation in acidic density, size and degree of aromatic structure, the water solubility and hence the mobility in the given environment of humic substances will vary to a large extent.

The major challenge in humic matter research is as in any environmental science discipline the great variability found in nature: Not only does any given sample of humic matter represent a complex mixture of substances collected from a complex physiochemical and biological matrix. The researcher would also have to deal with the huge spatial and temporal variation present at the given site and cope with the influence of a large number of environmental factors that may be of influence. Given this variation in the humic matter it self and in its surroundings, the research community faces a wide array of analytical and conceptual problems in its goal to understand the structure, nature and function of natural organic matter in the environment and the complex nature of these questions often call upon interdisciplinary cooperative studies.

Studies of structural information and physiochemical properties in humic substances are always conducted in order to better the understanding of the origin, fate and function of humic substances in the natural environment or in anthropogenic use. It is therefore essential that there: a) Exist a good overlap between the measured analytical parameter and the assigned actual physiochemical property, and b) that there exist a proper theoretical understanding of the link between physiochemical property of NOM and potential consequences of this property in the environment or anthropogenic use. This means that we can divide the scientific challenges into different levels or categories of problems ranging from theory and terminology generation to the actual understanding of humic matter structure, function and behaviour.

1. *Terminology and definitions:* What are the theoretical contents of the used definitions and terminology? Is the terminology good enough? Does it describe and distinguish between phenomena in a fruitful way for the scientific questions? Is it used consistently within the scientific environment?

2. Instrumental and analytical: How well is the analytical performance of methods and instruments? What are their uncertainties? Are these methods really suitable for the given problem? How can the methodology be improved to better serve the task?

3. Analytical interpretation: What do the results from a given method really tell about the humic substance? How well does it cover the physiochemical or structural property in question? Is the interpretation the same under all encountered conditions? Do results from several methods provide the same indications and if not, what does the difference mean in terms of physiochemical properties?

4. Link between physiochemical property and consequences: What do the measured properties indicate about the origin, fate and function of humic substances in the natural environment? How can different study approaches and strategies be combined to better the understanding?

These different levels of challenges will naturally interact with each other in a closed cycle of increased knowledge. A better understanding of humic substances in the environment, may lead to a better understanding of the structure and property of the material, and as new methods or procedures are developed and old ones improved and refined so are the possibility to draw safer conclusions about the behaviour of the humic matter in the environment. As the analytical and theoretic understanding of each level are refined, so are the possibility to discriminate between the natural variation within the humic matter itself, temporary and spatial variation and the pure instrumental and methodological uncertainty. Ultimately the new understanding may call upon a revision of the theory and terminology to better cover the problems and phenomena in discussion. The presented articles in this thesis are examples of such individual task falling into different levels of the analytical study and refinement within the humic matter research.

1.2 Article I: The merits of the high temperature combustion method for determining the amount of natural organic carbon in surface freshwater samples.

Safe and reliable measurements of the total concentration of organic carbon (OC) are essential in nearly all NOM research either it is used to monitor the natural variations in this parameter at natural locations or to interpret the results of other investigations like spectroscopic measurements, various fractionation methods, titration or NMR studies. Today the dominant method for the analysis of OC is by use of instruments of high temperature (600 to 900°C) combustion of OC to CO_2 over a catalytic material (usually platinum) followed by IR detection of the generated CO_2.

Problems with poor accuracy and precision in the analysis of organic carbon have been reported in several articles concerning both the marine- and freshwater environment (Perdue & Gjessing 1990, Benner & Strom 1993, Sharp 1993, Sharp et al. 1995, Sharp 1997). This situation has improved during the recent years, as the high temperature catalytic combustion analytical instruments, have become commercially available (Koprivnjak et al. 1995, Skoog et al. 1997, Sharp et al. 2002). However, these instruments were originally developed for the determination of the amount of carbon in samples from

oil refineries. The carbon analysis of NOM in water samples is today routinely determined using the operationally defined method intended for water samples contaminated with oil; i.e. non-purgeable organic carbon (NPOC). Although applicable also to natural samples, the merits from the manufactures do not necessarily reflect the analyte and matrix in natural aquatic samples.

Marine chemists have during the last decade documented and improved their analytical merits with great success (Sharp 1993, Sharp et al. 1995, Sharp 1997, Skoog et al. 1997, Sharp 2002). These achievements can however likely not be directly applied on freshwater NOM analysis, due to the structural difference between the freshwater and marine NOM (Stumm & Morgan 1981, Harvey & Boran 1985, Steinberg & Muenster 1985, Stevenson 1985), and difference in concentration range from that of marine NOM (Tait 1981, Perdue & Gjessing 1990). While the marine NOM is found in low total concentration and consist of smaller molecular entities with a high degree of aliphatic structure, the terrestrial and aquatically generated NOM is present in much higher concentrations (often orders of magnitude) consisting of material of higher molecular size and with a higher degree of aromatic structure. These factors cause the use of different high-temperature catalytic combustion material. Furthermore, the sample matrices differ greatly between the two environmental systems: The marine samples have a high salinity, while in the terrestrial and aquatic samples the potential generation of colloid material might pose an analytical challenge. A major concern in the analysis of freshwater NOM is the recalcitrant nature of this material (Perdue & Gjessing 1990). Potassium hydrogen phthalate is the common standard reference material prescribed by the International organization for standardization (ISO 1999). Because of its smaller size (MW 204.23 g/mol) and simple structure compared to average NOM, we hypothesize that it is likely to be easier to combust and thereby detect by these instruments than NOM. Method's merits generated using potassium hydrogen phthalate, may therefore not give a true picture of the method's ability to detect all of the more refractory fractions of NOM.

Standard RO-isolates from natural freshwater provides OC in a form that is easy to transport, store and handle, and which can provide samples with a controlled amount of OC. These materials have recently been made commercially available through the International Humic Substance Society (IHSS 2005). To our knowledge, no inter-laboratory comparisons concentrating on this method for determining of DOC in natural fresh waters and emphasising these potential problems have been published. Article I in this thesis reports the result of an intercalibration determining OC on a set of 10 fresh water samples conducted in August 1999.

1.3 Article II: Artefacts in XAD-8 NOM fractionation.

In 1976 Leenheer and Huffman published an operational defined chromatographic method for the fractionation of NOM into fractions based on their hydrophilic/hydrophobic and acid/basic character using a sequence of three different column materials (Leenheer & Huffman 1976, Leenheer 1981). There are now a large variety of modifications and clones from the original method of 1976, adapted to suit very different analytical purposes, on site fractionations and substantially larger volumes than the original method (Leenheer & Noyes 1984). The first column in the sequence contained the Amberlite XAD-8 resin from Rohm and Haas: A methylmethacrylate copolymer with weak polar properties and relative high

surface area. This material has over the last few decades also become a popular tool for isolation and subsequent investigation of the more hydrophobic fraction of NOM from an aqueous sample. This method was introduced by Thurman and Malcolm in 1981 and resulted in what they defined as a fulvic acid- and a humic acid fraction (Thurman & Malcolm 1981). The method was adopted by International Humic Substances Society (IHSS 2005) and the international standards and reference material of fulvic- and humic acids is based on isolation using XAD-8.

XAD-8 is widely used to fractionate NOM into hydrophilic and hydrophobic fractions by simply pumping a given volume of water sample at a fixed pH through the resin: The material retained on the resin is defined as *hydrophobic*, while the NOM that passes though the column is defined *hydrophilic*. The concentration ratio between the fractions is used to express the hydrophobic character of the NOM. As stressed in the original method development (Leenheer & Huffman 1976, Leenheer 1981), the XAD-8 technology provides an operationally defined chromatographic method of separation and investigation of the NOM material. The most important implication of this is that small differences in methodology may make comparison between data from various methods and operators difficult or even impossible. Several publications address this problem in critical reviews of the fractionation and isolation techniques (Aiken 1985, Aiken 1988, Leenheer 1985).

The XAD-8 methodology operates by the principles of chromatography, but the conditions of these methods are far from the ideal analytical conditions normally strived for in other chromatographic procedures (Skoog et al. 1988, Skoog & Leary 1992, Pole 2003). In more ideal chromatographic methods a small volume of sample is injected into the continuous flow of the mobile phase, the stationary phase is well dimensioned for the capacity of the applied sample volume, and the analytical components of interest are separated into defined peaks with good resolution. On the contrary; in the XAD-8 NOM fractionation methodology a complex mixture of substances with unknown physiochemical properties, representing a continuum of variation in affinity towards the stationary phase, is pumped in a continuous flow through a column that is relatively small compared to the size of the total sample. Any clear distribution coefficients of such complex mixtures are unachievable. At best an indication of an average distribution coefficient for the "average" humic substance in that particular sample with a specific concentration could be obtained. Furthermore the capacity of the XAD-8 towards NOM under these conditions could be a cause of concern. Peak separation and broadening cannot be defined, as there are no defined peaks to observe only an operationally defined cut-off between the so called hydrophobic and hydrophilic fractions. To complicate this further, the adsorption onto the XAD-8 material is highly pH dependent, which means that even small variation in pH may influence on the cut-off. Adding the experience that samples of NOM can be difficult to pH adjust precisely even at pH 2 because of relatively slow kinetics in this complex system, this renders a method that is highly vulnerable to the slightest change in analytical condition and the operators should therefore expect potentially poor selectivity and separation of the NOM on the XAD-8.

The central point of our work in this presented article is to challenge various assumptions on the performance of the XAD-8 methodology of NOM fractionation when analyzed under fairly common conditions. It is difficult to talk about standard conditions here since the variation in methodology within the NOM research community varies to such a large extent. During the preparations to this study a literature survey on articles covering the use of XAD-8 for NOM fractionation or isolation was conducted. 50 ordinary research articles covering the subject and published during the last decade in English written journals were picked out by

random selection. Given that about 50-100 articles are published each year on this subject, the 50 articles picked out represented approximately 5-10 % of the total published volume in this field. The articles were picked as random as possible, and not because they by any means represented neither significant studies nor bad examples. Thus an examination of these papers was likely to provide a good impression on how other researchers handle XAD-8 methodology when they conduct their studies.

Given the operational and highly chromatographic nature of the XAD-8 methodology, what was most striking during the survey was not what was written, but rather what was not written: The lack of detail in their experimental sections, lack of references to methodology, and lack of reflections on any bias caused by the methodology. In many cases the articles referred primarily to the work of Leenheer, Huffman, Thurman, Malcolm and Aiken (Leenheer & Huffman 1976, Leenheer 1981, Leenheer 1984, Thurman & Malcolm 1981, Aiken 1992) even when it was obvious from the presented study that this methodology was not followed directly. Some articles using XAD-8 as a tool for fractionation, referred to articles concerning XAD-8 as a tool for isolation and vice versa. Ten of the articles referred to previous work by the authors, but without providing sufficient basic information on methodology to interpret present article as such. In six articles no references to methodology was provided at all.

Frequently the definition of the methodology were limited to information of the type “passed though XAD-8 at pH 2” or “fractionated with the XAD-8 technique”. Most of the articles lacked key analytical information on column dimensions (and geometry), column-to-sample ratio, pump speed, DOC concentration of samples etc., information that could be critical in chromatographic procedures like the fractionation of NOM on XAD-8. Few articles provided information on blanks, replicate measurements and standard error in the given results, and given the time and volume consuming nature of these fractionations it could be tempting to give these questions low priority. The observations gathered during the literature survey do of cause not mean that all of these researchers are negligent in their performance of these methods. Article space is often of an essence, but given the complexity of these analytical operations and the amount of detail given for other procedures in the same articles, the lack of critical attention regarding the XAD-8 procedures is disturbing. Even though the work of the authors otherwise may be of high scientific quality and that they may well be aware of the potential artefacts of the methodology, the lack of vital information on a method of such a highly operational nature may put serious limitations on the readers possibility to understand, use, compare and criticise the results of such studies. This could also suggest that the researchers are unaware of potential limitations and artefacts and therefore are somewhat overconfident in the analytical performance of the method.

The aim of the work presented in this article is to search for methodical artefacts that may have influence on the interpretation of XAD-8 generated data and demonstrate the order of their influence. This was conducted by testing a set of hypotheses regarding the behaviour of NOM material and the XAD-8 materials performance and ability to separate hydrophobic from more hydrophilic NOM material:

1. Concentration effect: The ratio of the hydrophobic acid fraction (HPOA) to hydrophilic fraction (HPI) is independent of the original DOC concentration.

2. Chromatographic effect: The composition of the eluded HPI fraction will remain constant during the XAD-8 fractionation.

Secondly the questions of chemical change due to fractionation or storage of the fractions are addressed:

3. Stability and artefact essay: HPI and HPOA represent stable chemical defined fractions. Neither chemical alteration during the fractionation procedure nor artefacts due to chromatographic methodology will establish a new HPI/HPO equilibrium after a removal of either of the fractions.

In article II of this thesis laboratory experiments, using reverse osmosis isolates of NOM material, were conducted to test these hypotheses. Method reproducibility, especially in regards to different scaling of the methodical procedure and DOC concentration, as well as stability of the generated fractions is addressed.

1.4 Article III: The nature of the residual organic matter in drinking water following treatment with Chitosan and combined Chitosan / iron coagulants.

In the Nordic countries, surface water is often used for the production of drinking water. About half (w/w) of "the total matter" in many surface waters, is of organic nature. This natural organic matter has a characteristic brownish yellow colour. The coloured natural organic matter, as such, is not considered to be of any hygienic concern. However, there are five main arguments for why NOM should not be present in tap water.

- NOM have the ability to form strong complexes with inorganic and organic micro-pollutants (such as Hg, Cd, Cu, PAH, PCB). Thus elevated amount of NOM may also carry a high loading of micro pollutants into the tap water.
- NOM form disinfection by-products that may have mutagenic or carcinogenic properties (Rook 1976, Moore et al. 1978, Backlund et al. 1989).
- Part of the NOM is an energy source for micro-organisms in the distribution system, producing bio-fouling and most probably allergens and possibly myco-toxins.
- The colour of NOM may stain the fabrics during washing
- The colour is considered an esthetical problem

A commonly used method for removal of NOM is direct filtration after flocculation and precipitation with metal-based coagulants. However, some water works have found it difficult to comply with the low permissible levels of residual metal coagulant (aluminium in particular). Moreover, a high metal content in the produced sludge, leads to costly sludge disposal since it is considered to be a potentially harmful waste. The biopolymer Chitosan, produced from crustacean waste (i.e. shrimp shells), has been introduced as a promising alternative or supplement to metal-containing coagulants. Furthermore, the sludge produced with Chitosan is found to be more biodegradable than metal derived sludge. Chitosan has previously been shown to be an efficient colour remover. Based on the study of Vogelsang et al. (2004) it is shown that the Chitosan treatment preferably removes the largest molecular weight organic matter measured by size exclusion chromatography. On the other hand it is found to leave a higher proportion of the medium molecular weight organic matter in the treated water than the metal-based coagulants (Vogelsang et al. 2004). Co-addition of

Chitosan and iron chloride, however, has shown similar treatment results as the metal-based coagulants alone, but at a significantly lower total coagulant addition (Vogelsang & Liltved 2001, Vogelsang et al. 2004). The coagulating effect of Chitosan on NOM is due to a combination of charge neutralisation and inter-particle bridging (Vogelsang et al. 2004). Due to the potential problems of residual NOM, as indicated above, a better characterisation of the properties and stability of the remaining organic matter (OM) after coagulation with Chitosan is needed.

The presented work of this article report on the physico-chemical characteristics of residual OM in raw water after treated with Chitosan. Furthermore, the structural changes that apparently take place in the residual OM upon storage are studied. XAD-8 fractionation and UV-, visual- and fluorescence spectroscopy have been used to elucidate the characteristics of the remaining OM. The purpose of this study has also been to document if the most successful NOM removal regime is achieved by using Chitosan treatment alone or in combination with metal coagulants.

This study addresses the structure of the residual NOM by the means of different analytical approaches. The primary aim of the study was to gain information about the structure and physio-chemical properties of the residual NOM focusing on the practical and chemical suitability of Chitosan based coagulation for the purification of drinkingwater. Secondary, in the context of analytical challenges addressed in the general introduction, it is also interesting to discuss how the different characterisation methods offer an agreement in the interpretation of the NOM structure. In this aspect it may be vital to define what the purpose of the investigation is: What characteristics of the NOM and possible consequences of these characteristics the study is in search for. What do we mean by hydrophobic/hydrophilic characteristics of NOM? Are we searching for the material's potential for mobility or aggregation or are the main focus in this case the biological availability of the material? All these aspects are linked to what we consider to be the hydrophobic/hydrophilic behaviour of the NOM. And although spectroscopic methods may provide indications on the size and aromatic character of the NOM, these features may not overlap completely with the consequences of the higher order of structure that the XAD-8 may provide information on.

1.5 Article IV: The effect of forest liming on humic mobilization and physicochemical properties.

Large forested areas of the southern and western parts of Norway have for several decades been affected by acid deposition, resulting in acidification of lakes and streams, and substantial damage to fish populations and aquatic ecosystems (Henriksen et al., 1999; Hesthagen et al., 1999). Liming with calcite or dolomitic limestone has been used to improve the water quality and thereby prevent some of the damage to lakes and rivers. Terrestrial whole-catchment liming has the advantages over the direct aquatic liming of lakes and streams that it attacks the root of the problem; Decreased base saturation (BS) of the soil, caused by the acid rain, along with elevated levels of mobile acid anions enables leaching of H^+ and toxic inorganic aluminium to surface waters (i.e. water acidification). Terrestrial liming increases the BS of the soil allowing the pH of surface waters to increases along with a decrease in inorganic aluminium species. The terrestrial liming will have a more long-term effect and thereby represent a low-maintenance amelioration alternative.

A Norwegian research program "Counteractions against acidification in forest ecosystems" was launched in 1991. As a part of this research program, a whole-catchment liming experiment was conducted in a coniferous forest in Gjerstad in 1994 in the southernmost Norway (Hindar et al., 2003; Hindar, 2005). Two comparable catchments were selected, one to be limed with dolomite and one to serve as a control catchment. Water chemistry in the streams draining the two sites was monitored for one year prior to the liming in 1994 and then eight years post liming. The Gjerstad catchments have also been included in the Norwegian "NOM typing project (Gjessing et al.; 1999), in which reverse osmosis isolates of natural organic matter (NOM) from eight catchments in Norway were subjected to a large number of chemical and structural analyses.

Major changes in the surface water chemistry after liming was described and discussed by Hindar et al. (2003). Among these is a significant increase in pH, decreased concentrations of inorganic aluminium, along with an increase in the TOC concentration. Several mechanisms could govern this increased leaching of TOC: a) increased terrestrial production, b) increased micro-biological degradation or c) mobilization of less soluble terrestrial NOM.

The work of this article explores further the mechanisms behind this elevated mobilization and possible consequences of liming on the NOM structure and properties. The mobility of NOM is depending on the solubility of the substance, and all features of NOM that increase the water solubility will also influence on the potential mobility. This solubility is naturally highly dependent on the outer ruling conditions (matrix dependent), like pH, concentration of other ions, clay content etc., but also four major NOM characteristics that determine the solubility can be pointed out in what is likely to represent the order of importance:

- Free charge density: The number of charged sites divided by the total molecular mass will have the largest impact on the water solubility of the humic entity whether it is considered a molecule or a colloid. The charged sites of NOM are mainly negative sites deriving from the acidity of phenolic and carboxylic groups. This means that the main driving force in humic matter mobility is highly dependent on pH and in some cases also the present concentration of bi- and trivalent cations that may complex and precipitate the organic matter.

- Total density of functional groups. The total frequency of functional groups with available electron pairs (e.g. acids, alcohols, nitrogen- and sulphur- carrying groups) whether protonized or not, may offer the possibility for hydrogen bonding with water and though that stabilise the molecule or colloid in the water phase.

- Total size of the NOM entity: Smaller molecules or colloids are generally easier to dissolve or dispend in water than large ones.

- Ratio between aromatic and aliphatic structure: It is a general trend in organic chemistry that aromatic structures are considered to be more hydrophobic than aliphatic structures given that both are uncharged and of the same molecular size. There is little reason to believe that the larger humic structures should differ from this trend.

The effect of the two last points may be difficult to distinguee from each other as they are often found to be strongly correlated. The larger entities of humic matter are usually found to be more aromatic in structure and also more hydrophobic. More hydrophobic entities might also have a more significant tendency to form aggregates and colloids and though these processes further increase in size. Mechanisms of self-assemblance of humic matter aggregates are found in literature (Shinozuka & Lee 1991, Wershaw 1999, Guetzloff & Rice 1994, Tombácz 1999). Such self-assembly processes must represent equilibrium between formation and spontaneous destruction of the aggregates with kinetics slow enough to allow stronger and more permanent bonds to be formed. Hence the formation of NOM aggregates will depend on physiochemical conditions of the solution as temperature, pH, ionic strength and concentration of inorganic species, as well as the concentration and physiochemical properties of the humic matter itself. A low concentration of humic material would make the formation of larger aggregates less likely than a high concentration. Furthermore humic material with a high ability to form aggregates of increasing density would express higher probability to form and maintain growing aggregates and thereby obtain higher observed average molecular size, than materials with lower density packing order. These physiochemical properties can be related what commonly is considered to be the degree of hydrophobic nature found in humic substances, and it can therefore be linked to studies including XAD-8 fractionation analysis, size fractionations, NMR- and spectroscopic studies or octanol-water partitioning coefficients.

Humic matter of terrestrial origin that derive mainly from lignin as a substrate, are naturally found to be more aromatic, of a larger average size and less soluble nature than the aquatic generated counterparts. The transport of NOM through a catchment from the terrestrial environment to the stream and further on into the aquatic environment can in fact be viewed as a giant, natural chromatographic process with a continuous change in chromatographic conditions. Thus at any given point in time and ruling outer conditions, it will always be the humic entities with the highest solubility that has the potential for travelling at the highest speed with the provided speed of the water flow though the catchment.

In this presented article two very different research strategies are combined: A long term data serie from the monitoring of a standard set of few but significant parameters over nearly a decade, and a large collaboration study focusing on a large number of characteristics of two standard NOM materials (RO-isolates) from the same sites. The main analytical challenge of this study was to investigate to what extent the combination of these very different scientific approaches could be combined to reveal any mechanisms behind the increase in TOC observed after the experimental terrestrial liming of the Gjerstad site (Hindar 2005) and whether it could leave clues about the consequences for the structure and physio-chemical properties of the mobilized NOM material after the liming.

2. Materials and methods

2.1 Material

Artificial samples and synthetic humic material

Two of the studies in this thesis use artificial samples or synthetic humic material to test methods under very reproducible conditions. These synthetic substances have a structure that resemblance and therefore mimic the behavior of natural humic acids to a certain degree.

In article I; the NOM high-temperature catalytic combustion intercalibration study, several artificial sample materials relating to the method was used along with the natural freshwater samples and RO-isolates to test various aspects of the methodology. Potassium bicarbonate was chosen as the standard for measuring inorganic carbon. Potassium hydrogen phthalate is considered as a reliable and easy combustible standard material for the high-temperature catalytic combustion method, and was used by the majority of the participants in the intercalibration as their reference material used for preparing OC standards. This material was therefore chosen as the safest reproducible material and used in artificial samples at different concentrations in order to test the reliability of the method from low to high carbon concentration under optimal conditions. In addition an artificial standard was made from Cu-phthalocyanine-tetrasulfonic acids, a material known to be more difficult to combust. This standard was chosen to represent a greater challenge to the instrument and mimic the more recalcitrant humic material.

In article III; NOM coagulation treatment with Chitosan, the raw-water was prepared from a synthetic humic acid with 39% C content supported by Sigma-Aldrich (Humic acid sodium salts, Sigma-Aldrich Chemie). The organic matter content of the water was designed to reflect the humus rich waters often used as raw water source for the production of drinking water. This synthetic humic material was chosen over natural humic matter for two reasons: 1) to represent an initial pilot experiment performed under controlled and reproducible conditions, and 2) to be integrated into a larger project on Chitosan coagulation treatment initiated by The Norwegian Institute for Water Research (NIVA) and comparable to other experiments in this project already using this artificial standard (Vogelsang et al. 2004).

The great advantage of synthetic humic acids or artificial standards is the defined composition that enables repeated experiments under identical conditions with an unlimited substrate source, and the more defined structure might aid the understanding of mechanisms. Their greatest disadvantage is of cause that they are not real humic acids, and therefore lack the ability to predict the full behavior and complexity of natural humic substances collected under various conditions. These substances are therefore best used in research pilot experiments or as a supplement to natural humic matter.

Freshwater samples of natural organic matter

The freshwater samples used in article I (intercalibration study) and article IV (effect of liming) originates from surface water collected at Nordic experimental sites. The fresh natural water samples of the intercalibration study (article I) were collected from stream or lake water in three of the Nordic catchments studied in the NOMiNiC project (Vogt et al.

2001). In order to minimize changes in the amount of TOC during shipment and handling the bulk samples were all filtered using a ~0.7 μm pre baked Whatman glass microfibre filter (GF/F 47mmØ) prior to shipment to the participants of the intercalibration study. The participants were instructed to store the samples cool and conduct the analyses for TOC, DOC and DIC within a week using their own filtering equipment to define the cut-off between TOC and DOC and their own method for DIC determination. In the case of the liming study at Gjerstad sites (article IV), samples were collected every two weeks from the two streams from May 1993 to June 2002. All samples were analysed 2-3 days after sampling for major chemical constituents and Al-fractions. Water flow in the two streams was monitored at calibrated 120° V-notch weirs (Hindar et al., 2003). All reported data were volume weighted results.

The greatest benefit using natural water samples in NOM research is that they represent NOM as found in its most natural form and in what can be assumed to be closest to its original matrix. Even the gentlest form of isolation into a manipulated concentrate or dry sample can not offer any guaranties against permanent change in structure or physiochemical properties of the original NOM. The largest disadvantage of natural samples is their vulnerability towards change during storage and handling. Samples filtered at 0.45 μm may still undergo biologically induced changes, as heterotrophic bacteria are small enough to pass through filters of this pore size. NOM degradation and consumption by heterotrophic bacteria are well documented in literature (Jones 1992, Tranvik 1992, Steinberg 2003). Not even samples filtered at a smaller pore size (e.g. 0.22 μm) or samples conserved to specifically limit biological activity can be assumed to be totally free of biologically induced change, as the exo-digestive enzymes released by bacteria to digest organic matter may be present for a significant period even after the bacteria itself are eliminated. Conservation of natural samples, usually performed by adding substances toxic to the micro organisms (often small amount of heavy metals like copper), may introduce new problems since it could interfere with analytical methods later applied to study the NOM. In case of the natural samples of the studies presented in this thesis, not initial conservation schemes were used.

Reverse osmosis isolates of natural organic matter

Reverse osmosis isolates (RO-isolates) was used as samples in the experiments described in three of the studies presented in this thesis. These RO-isolates were chosen from sets of RO-isolates available from two projects; the Nordic NOM typing project (Gjessing et al., 1999) and the NOMiNiC project (Vogt et al. 2001). The RO-isolates from there projects originates from surface water samples from Nordic sites of various characteristics and are well described through multidimensional analysis and structure studies from international collaboration projects.

In the main procedure of RO-isolation (Serkiz and Perdue 1990), humic matter from a large volume of surface water (typically 1000-3000 L) is isolated. The water is pre-filtered (1μm) before it is pumped through the reverse osmosis unit (about 150 Å) *in situ*. A cation exchange unit replace all cations with Na^+ to prevent precipitation of insoluble salts. The resulting humic concentrates (typically 25-50 L) are then filtered through 0.45 μm filter before transport to the laboratory. In the laboratory each of the samples are further concentrated by a rotary evaporator to a volume of about 5 L and then finally freeze-dried.

Humic standards isolated by reverse osmosis (RO-isolates) have become increasingly popular as a research tool over the last decade. The material is produced from large amounts of surface water which enables the isolation of relatively large amount batch samples. In its final form the RO-isolate is a dry powder; easy to store, ship and handle, and that can be easily re-dissolved in variable concentrations to solutions of low ionic strength. The RO isolation procedure is also considered to gentle to the humic material compared to many of the earlier isolations methods (e.g. XAD-adsorption/desorption, acid precipitation), and therefore more likely to reflect the true structure and characteristics of natural water samples. Among the main possible disadvantages of the RO-isolates it should be noted that although the isolation procedure is considered to be gentle, no guaranties can be given that the characteristics and structure of the dry isolate resembles that of the original sample. Each individual RO-isolate is also represented by a batch sample, which puts a final limit to the available amount of substance of similar characteristics. The method is also unsuitable for the isolation of certain types of humic matter, like terrestrial humic matter from soil or soil solution simply because it is difficult obtain in sufficiently large solution volumes.

2.2 A short description of the research sites related to the studies.

Scandinavia can today offer a large number of research sites suitable for NOM research. These sites are all thoroughly studied research sites, well documented through long term international and collaborative interdisciplinary research programmes, among them the HUMEX project (Gjessing 1992, Gjessing 1994), the NOMiNiC project (Vogt et al. 2001), and the NOM-typing project (Gjessing et al. 1999). In addition to the large supply of fresh water samples from soil solution and various surface water qualities collected at these catchments, these catchments have also provided a large number of standardized RO-isolates (20+) of which many are commercially available or distributed through international collaborations to fellow NOM researchers. These isolates are now well documented and offer a large span in NOM structure and physiochemical properties in accordance to the large variety of the original site characteristics, and they therefore have the potential to suit many different scientific purposes. All NOM from freshwater samples and RO-isolates presented in the articles of this thesis originate from Scandinavian research sites, selected individually to represent NOM of different qualities: Lake- or stream water, fresh or refractory material, more or less terrestrial or aquatic in nature, from pristine, acidified or limed catchments.

The site of *Birkenes* (Mulder et al. 1999) in Southernmost Norway about 30 km north of Kristiansand, at an altitude of between about 190 and about 310 m above sea level. The catchment covers an area of 41.6 ha and consists of 2 main valleys; the lower valley with the main stream being Vestre Tveitdalen and the higher valley with the main bog and a slower flowing tributary stream being Langemyrdalen. These two streams, together with a third small stream, converge about 150 m above a V-notch weir. This is a well studied anthropogenically acidified site with low pH and high ionic strength. Samples from the stream of this site are consisting of NOM under long-term acidic influence, which may influence on the mobility and thereby the quality of humic matter reaching the stream.

Not far away from the Birkenes site, the *Gjerstad* experimental site (Hindar et al., 2003) is located in Aust-Agder County in southernmost part of Norway. This is another site heavy influenced by long term anthropogenic acidification. In this site a paired liming experiment

was conducted, where one catchment (84 ha) was selected for liming and a nearby catchment (41 ha) served as a control catchment. Both catchments of the Gjerstad experimental site are forested with mixed coniferous forests. Soils are organic rich acidic podzols. This soil type is commonly found in forests on the nutrient poor siliceous moraine in southern Norway.

The *Skjervatjern* site established in 1987 as a research catchment for the HUMEX project (Gjessing 1992, Gjessing 1994) is located near the western coast of Norway, about 10 km north east of Førde. The site is relatively small covering an area of 11.3 ha inclusive a small humic lake. The catchment consists of a mixture of histosols and mire, mineral soil (podzol), shallow soil profiles and bare rock. The lake is nearly surrounded by dystrophic, water clogged sphagnum mire on all sides. The lake is feed through seepage through the mire and underground vents running through the mire. The level of sulphate deposition is low to medium compared with acidified catchments in southern Scandinavia (e.g. the Birkenes catchment). From 1988-1996 this catchment was studied intensively in the HUMEX acidification research program. During this period the lake and catchment were divided, and one side was sprinkled with artificial acid rain. Even though the soil profiles in the Skjervatjern catchment are relatively shallow, the catchment revealed a good buffer capacity and only small changes in the water chemistry were found. Presently, after the conclusion of the acidification experiment, water samples collected at the lake exhibit a medium high NOM concentration (approx. 4-6 mgC/L), fresh material with relatively high colour and buffer capacity.

The site of *Hellerudmyra* is a site subjected to long term study of NOM. The catchment (8 ha) is located west of Oslo in a relatively isolated area. The main feature of the site is a large bog surrounding a small dystrophic lake (700 m^2). The pond of this catchment has provided reference water serving a large fraction of the NOM research conducted in Norway during the last four decades. Samples from the site provide water with very high DOC concentration (10-25 mgC/L) and low natural pH (pH 4-5), and the NOM of the samples exhibit a high concentration of fresh organic material with high relative colour. Several RO-isolates have been collected from this site. Furthermore the Nordic humic and fulvic standards isolated by use of XAD also originates from this catchment. These two standards are commercially available from the IHSS (IHSS 2005).

Lake Maridalsvann which is a large lake (3.9 km^2), is situated just a few km north of Oslo. Besides being the main water supply for the city of Oslo it has also its merits as a study site in NOM research. The actual connection to drinking water makes it an interesting candidate for studies covering the subject humic substances in surface water used for water supply. Water samples from this location generally exhibits a low concentration of organic material. The NOM from this location is considered to be of refractory nature (Vogt et al. 2001).

The *Hietajärvi site* (Bergström et al. 1995, Bergstöm 1998) is located in North Karelenr of Finland. It includes a large lake (1.12 km^2) probably causing the sample to consist of NOM with a high degree of refractory material in a high pH environment.

The *Svartberget site* in Northern Sweden (Grip & Bishop 1990, Bishop et al.1994) consists of a dystrophic stream draining a large bog. This stream provides samples of high total NOM concentration and consists of less complex fresh but relatively hydrophobic humic material.

2.3 Analytical methods

NOM high-temperature catalytic combustion

OC were determined in using a high-temperature catalytic combustion with an IR detector. At the University of Oslo, Department of Chemistry this is a Shimadzu TOC 5000A analyser (agreement within 0.2 mgC/L) (Shimadzu 1998). This was also the most popular brand of instrument at the laboratory participation in the intercalibration study. In all these instrument OC is combusted to CO_2 by means of high temperature and catalysis. The CO_2 can then subsequently be detected using an IR detector. Combustion temperature of the instruments presented in the intercalibration study varied from 680 to 900°C. Platinum, and in some cases palladium, is used as the catalytic material. Most of these instruments can be equipped with catalyst material of different sensitivity. A high sensitivity catalyst typically provides reproducible results well below 1 ppm and would probably be the natural choice for marine NOM analysis with concentrations rarely exceeding 2 ppm. The concentrations normally found in freshwater, would soon devour this catalyst. The freshwater chemistry environment is therefore limited to a less sensitive catalytic material with higher capacity, which is suitable for analysis above 4-5 ppm, and has a reliable detection limit in the area of 1 ppm (Shimadzu 2000). The reactor chamber found in the instruments of the partitioning laboratories of the intercalibration study varied in size and geometry, with a length from 8 to 44 cm and diameter 4 to 22 mm, placed vertical or horizontal in the instrument. The carrier gas was either synthetic air or O_2.

UV-VIS spectroscopy

Unless otherwise specified, all samples were acidified to pH 2.0 (± 0.1) with 37 % HCl just prior to measurements, as the absorbtivity is known to be pH dependent (Tsutsuki & Kuwatsuka 1979). pH 2 was selected since this is the key reference pH of the XAD-8 procedure. Re-measurements of a random subset of samples revealed no significant or systematic change in absorptivity over a 48 hour period. Sample measurements were blank-corrected by subtracting the spectrum of each sample with the spectrum of carbon free water (Milli-Q185, Millipore 2004).

Two instrumental modes for the measurements of UV-VIS absorption are used in the studies:

Mode 1: Absorption measured on a Hitachi U2000 Spectrophotometer at 254, 400 and 600 nm using a 1 cm quarts cell. Specific Absorption Ratio (SAR; A_{254nm}/A_{400nm}) and the specific UV absorption (sUVa = $A_{254nm}/ mgCL^{-1}$) were calculated as indicators of structural differences between compared samples.

Mode 2: UV-visible absorbance scans between 190 and 1100 nm performed on an Agilent 8453E photodiode array spectrophotometer with accuracy of ± 0.005 absorption units (A.U.). The absorbance at 254 and 400 nm were used to calculate sUVa and SAR. Since samples of the Chitosan study (article III) displayed a relatively low total absorbance at 400 nm, a 300 nm version of "SAR" were calculated for the whole 250-600 nm spectra ($SAR_{300} = A_{\lambda}/A_{300nm;\{\lambda \in 250\text{-}600nm\}}$) in order to verify any blue- or red shift throughout the spectra using a more significant reference wavelength.

The chromophores in NOM that are expected to absorb light most strongly are π-conjugated systems, commonly found in complex aromatic compounds (William & Flemming 1980). Increased length of conjunct double bonds is known to shift the absorbance towards longer wavelength due to increased resonance of the molecular structure (Fessenden & Fessenden 1978). An increased *red shift* (i.e. reduced specific adsorption ratio (SAR = A_{254nm} / $A_{400\ nm}$) is therefore, in the context of humic substances, associated with more hydrophobic and larger molecular structures (Abbt-Braun & Frimmel 1999, Malcolm 1989, Thomsen et al. 2002, Andersen et al. 2000).

Fluorescence spectroscopy

Fluorescence measurements were performed on a Perkin-Elmer LS-50B scanning spectrophotometer operated in the emission scan mode at 22 ± 2°C using matched 10 mm quartz cuvettes. Emission spectra were recorded between 380 and 550 nm at an excitation wavelength λ_{ex} = 360 nm. The wavelength range of the emission spectra was chosen as dissolved aquatic NOM (DNOM) is known to exhibit an emission maximum in the region 410 - 490 nm (Senesi, 1990). An excitation wavelength of 360 nm seems from literature to be favoured in fluorescence emission studies of DNOM (Senesi 1990, Senesi et al. 1991, Miano et al. 1992). The emission spectra were corrected for inner-filtration effects by multiplication with the factor e^A, where A is the sample absorbance at the excitation wavelength (Zsolnay et al. 1999). The spectra were subsequently used to calculate the Humification Index (HiX) as given in equation 1. In this study HiX was calculated to determine whether or not a preferential removal of high-Mw structures had occurred upon the different treatments. A decrease in the HiX of the DNOM, corresponding to a blue-shift, would be indicative of such preferential precipitation. Relative fluorescence spectra is obtained by dividing the total fluorescence by the concentration of carbon (mgC/L) in the sample

Eq.1 $HiX = I_{465\text{-}540} / I_{390\text{-}465}$ (I = emission intensity at each wavelength)

XAD-8 fractionation of NOM

The procedure for NOM XAD-8 fractionation used in these studies is developed from the basis of work of Leenheer and Huffman (Leenheer & Huffman 1976, Leenheer 1981). A full operational procedure for this fractionation method as used at the University of Oslo (Environmental Chemistry Group) during the resent years can be found in Appendix A.

Unless otherwise noted the XAD-8 fractionations were performed by fractionating 180 mL sample through a XAD-8 column (length 8 cm and inner diameter 5 mm; 3 mL resin volume) at a flow speed of 1 mL/min. Prior to the fractionation the sample was acidified to pH 2.0 (± 0.1) by addition of conc. HCl (p.a.). The whole sample was then without delay pumped through the XAD-8 column. The hydrophobic fraction, (HPO), is adsorbed by the XAD-8 material and the hydrophilic fractions (HPI) pass through the column. The hydrophobic acid fraction (HPOA) was eluated by back-flushing the column with 1 N NaOH solution at a flow speed of 1 mL/minute. 30 mL of eluated HPOA was collected and then diluted back to its original concentration by adding 150 mL carbon-free water. The XAD-8 fractions (HPI, HPOA) were stored in pre-baked brown glass bottles in the dark at 4 °C until analyzed or further fractionated. The hydrophobic neutrals (HPON) are defined as the fraction that remains on the XAD-8 column after the NaOH extraction. The size of this fraction is obtained by subtracting the amount of carbon in the HPI and HPOA fractions from the total sample

DOC. HPON compounds are removed from the column by a washing procedure using NaOH, HCl and carbon free water between the samples. Blind samples of carbon-free water were treated and run as an ordinary sample frequently in between the ordinary samples. These blind samples revealed a background DOC leakage from the column ranging from 0.1-0.3 mgC/L for the HPI fractions and 0.2-0.5 mgC/L for the HPOA fractions (depending on the column and number of runs performed on it). These background values are subtracted from the sample fraction DOC.

Octanol-water partitioning coefficient (Kow)

The octanol solubility on RO-isolates reported in article IV was recorded by Gjessing and co-workers (Gjessing et al. 1999). The RO-isolates were dissolved in carbon free water to an approximate concentration of 5 mgC L^{-1} (10 mg organic matter per L, ash content accounted for). Aliquots of these solutions were pH adjusted with HCl to pH 1, 2 and 3. Octanol was added (5 mL octanol to 15 mL sample) and this mixture was carefully shaken for 150 min. The distribution of TOC in the water phase was determined by measuring the absorption at 254 nm.

NMR

Liquid state ^{13}C NMR and ^{1}H NMR measurements presented in article IV were recorded by Pempkowiak (2005) using a Varian Plus 500 NMR spectrometer at 125 MHz (0.2 mol/l NaOD in D_2O). The ^{13}C NMR spectra were recorded in solutions containing 100 g L^{-1} of RO-isolate. The probe temperature was 20 °C. The acquisition conditions were as follows: Acquisition time 0.3s; relaxation delay 5.0s; spectral window width 57544 Hz; number of scans 42000. Proton decoupling mode was utilized. The ^{1}H NMR spectra was recorded under the same instrumental and analytical conditions except that the sample contained 20 g L^{-1} RO-isolate and that the number of scans was 5000.

Proton binding capacity – titrations.

Proton binding capacity of the RO-isolates presented in article IV were analysed and modelled by Takács et al. (1999). The RO-isolate were dissolved to original sample concentration in 0.05 M LiOH to avoid alterations of the concentration of major cations, and shaken under nitrogen for 24 h. The solutions were filtered through 0.2 µm glass fibre filters and adjusted to pH 8 with HNO_3. Ultra filtration was carried out on the DNOM sample solutions through 1000 Dalton molecular weight filter. Each sample was filtered twice and an aliquot of the >1000 Dalton retentate was used for the titration. The samples (15.00 mL) were diluted with 50.00 mL 0.05 M NaCl and acidified to pH 3.5 with 0.1 M HCl and purged with nitrogen. Equilibrium titrations were made twice with 0.05 M NaOH to pH 11 with fixed increments (0.1 mL). Equilibrium back titrations were made with 0.1 M HCl to pH 3.5 with fixed increments (0.05 mL). Interpolation of the fixed titration increment profiles was performed on a fixed titration increment grid (Δ pH 0.5). The pK_a values were determined with Linear Programming with 0.1 pK_a grid, using the pH intervals 4.0 – 4.9, 6.5 – 7.4 and 9.0 – 9.9.

2.4 Statistical tools.

In the four articles presented in this thesis, a large variety of statistical tools, from the simple calculation of average, standard deviation and T-tests to more sophisticated statistical methods, are applied in order to test hypothesis and significance of results. Unless otherwise noted the level of significance is based on a 95% confidence level. Statistical tools are used in all of the studies but were used most extensively in article I (The TOC intercalibration study). In order to identify outliers and significant differences between data populations, accuracy and precision, and performance of the participating laboratories, various statistical tools both assuming normal distribution and non-parametrical ranking methods, have been applied. Limit of Detection was calculated according to the recommendations of Miller & Miller (2000) and Skoog et al. (1988): i.e. LOD = t-value ($p = 0.01$, $N = 16$) · std of blank values from all the laboratories.

In the intercalibration study outliers were detected at 95% and 99% significance both using Students T-test and the Dixon Q-test. The 95% significance test should be interpreted with care since statistically one of twenty results will lie outside the 2.5% borders. In the population of 25 participants it was therefore likely that the value from one laboratory would lie outside the borders for random reasons. However it is not likely that the same laboratory is represented several times in this range. Some of the laboratories were over-represented in the number of outliers.

A large amount of the data from the intercalibration study were not normally distributed. E.g. the overall analytical performance found of the individual intercalibration samples was difficult to analyse with parametric methods because of the large span in concentration and standard deviation of these samples and that the tendency of over- or under estimation of some laboratories causing a non-normal distribution. A non-parametric ranking test was therefore applied. In this method the OC results on each sample were sorted and the laboratories were assigned a ranking number for every sample according to their sequence in an increasing concentration order. The total score or sum of ranking from the different samples or tests is then compared. If the differences in results are caused by random errors, the sum of ranks should be normally distributed around a middle score even if the parameter it self is not. That is, it is unlikely that a laboratory will score very high or low on every sample and this suggest that that the candidate consequent over- or underestimate the OC content of samples. The test can also be used to rank the candidates in accordance to how far they are from the median value for any given sample. The score will thereby suggest which candidates that are most likely to have a generally poor precision. The score indicating any specific significance level can be obtained from the normal distributed theoretical score of ranking.

Youden analysis (Youden 1959, Youden & Steiner 1975) plots of the intercalibration data provided useful visual illustration for essential features found in this intercalibration. The individual results were normalised by dividing with the average result or, when known, the actual concentration, and subtracting 1.

A large amount of the information provided by the participants of the intercalibration study was non-value indicators in groups of low and variable numbers. The influence of these parameters is investigated by simple non-parametric ranking tests for significant differences between various analytical parameters (e.g. instrument type, reactor temperature, filter type etc.). The individual influence of these factors on the results could then be analyzed. Non-parametric ranking tests can be useful when for analyzing data divided into small and un-

evenly sized groups, because of the robustness of these tests. They are however not very specific and need a high degree of difference between populations to indicate significance.

3 Experimental concepts, results and discussion

3.1 Article I: *The merits of the high temperature combustion method for determining the amount of natural organic carbon in surface freshwater samples.*

The intention of the intercalibration was to reveal problems when determining the amount of carbon of NOM in fresh waters using high temperature combustion TOC analysers. The intercalibration samples were therefore selected to satisfy the following requirements:

A. Certified concentration for accuracy determination
B. Non-labile material in order to assure no change during sampling and handling
C. Contain not readily oxidizable material in order to reveal inadequate ability to combust all fractions of NOM
D. Cover the range in [TOC] and matrices commonly encountered in natural samples
E. Low concentration in order to reveal detection limit and high blank value problems
F. Mix of organic and inorganic carbon in order to reveal deviations that can arise by the use of various techniques in determining total carbon (TC), total inorganic carbon (TIC) and total organic carbon (TOC).

The samples that were used for the intercalibration are presented in Table 1. The intercalibration results of the combination of these eleven samples were found to be well suited for discussion of problems associated with the determination of carbon in NOM found in freshwater locations.

Table 1: Description of the samples used in the intercalibration. The letters in the Purpose column refer to the list of requirements stated above.

Sample type	Description		Purpose	mgC/L
RO-isolated Na-salts	1	The Nordic reference NOM	A, B	9.4
	2	Hellerudmyra (Spring), Eastern Norway	B, D	18
	3	Lake Maridalsvann, Eastern Norway	B, C	2.7
Fresh Natural water	4	Lake Hietajärvi, Eastern Finland	C, D	4.1
	5	Svartberget, Northern Sweden	D	15
	6	Birkenes, Southernmost Norway	D	8.0
Synthetic Standards	7	Potassium hydrogen phthalate	A, E	0.5
	8	Potassium -bicarbonate and -hydrogen phthalate	A, F	9.0
	9	Potassium hydrogen phthalate	A	9.0
	10	Cu-phthalocyanime-tetrasulfonic acid	A, C	9.0
	11	Local carbon free water	E	0

The samples were sent as freshwater samples or in case of the RO-isolates as dry samples in sealed ampoules. Along with the samples the participants were also provided with instructions on how to dissolve the RO-isolates and provided with dedicated filtering equipment

(Millipore MILLEX-HA non-pyrogenic sterile 0.45 µm filter cartridge using a 5mL syringe) for the RO isolates in order to distinguish loss in precision due to the filtering step from measurement errors in the data set. The participants were informed how to rinse these filters prior to filtration to avoid contamination. The sets of water samples (no. 4-7) and standards (no. 8-10) were distributed in pre-baked brown glass bottles with teflon-lined caps using express-mail. The individual laboratories were requested to prepare and determine their carbon free water, which is the eleventh sample. The total set of samples was followed by general instructions on how to conduct the intercalibration and a questioner asking for specific questions regarding the instrumental conditions and standard operational procedures of the laboratories. The participants were encouraged to use their laboratories own normal procedures regarding filtering, conserving and standards.

After completing the analysis the participants were requested to submit their results together with their own standard operational procedure (SOP) and the questionnaire, and send back aliquots of the leftovers from the 11 samples for re-analysis of TOC and UV-absorption at the University of Oslo. This re-analysis revealed no significant changes in the OC content. This could be interpreted as any possible changes to the samples happened prior to the shipment to the participants, and that the samples were fairly stable after any initial changes. Table 2 summarises the general statistics for the analysis of the intercalibration samples. The absolute standard deviation (STD) between the laboratories (0.3 - 1.4 mgC/L) was nearly one order of magnitude larger than the deviation between the three replicates of each laboratory. The best reproduction was found for the standard material.

Table 2: General statistics of the intercalibration results. Numbers 1-10 refer to the intercalibration samples described in Table 1. Values are given with the denomination mg C L^{-1}. Outliers at 95 % significance level are removed from the general statistics.

	RO-isolates			Fresh natural samples						Synthetic standards				Blank
	1	2	3	4	4	5	5	6	6	7	8	9	10	11
	DOC			TOC	DOC	TOC	DOC	TOC	DOC	TOC				TOC
MEDIAN	8.6	17	3.0	4.2	4.1	15	15	8.1	4.1	0.7	4.7	9.0	11	0.1
MAX	9.6	19	3.8	5.5	6.2	17	17	8.8	6.2	1.2	6.0	9.6	13	0.8
MIN	7.0	13	2.4	3.1	3.1	12	13	6.9	3.1	0.0	2.8	8.2	9	-0.1
Outliers	1	1	2	0	1	2	2	2	1	0	1	0	2	-
AVG	8.5	17	3.1	4.3	4.2	15	15	8.0	4.2	0.7	4.8	8.9	11	0.1
STD	0.71	1.3	0.38	0.61	0.70	1.2	1.4	0.48	0.70	0.27	0.62	0.41	1.3	-
SDT%	**8.4**	**7.3**	**12.5**	**14.3**	**16.4**	**8.2**	**9.5**	**6.0**	**16.4**	**38.8**	**13.1**	**4.6**	**11**	-
N	24	23	22	25	23	23	22	23	23	23	24	25	23	18

The general conclusions are that samples near the level of detection showed a tendency to be overestimated, while samples of high OC concentration and samples believed to be of refractory nature were underestimated In some cases only 30% of the OC in the sample was detected. The only coherent difference between laboratories with systematic too low estimates and the rest of the participants was that the reactor chamber in their instrument was placed horizontally instead of vertically. A horizontal reactor tube may serve as a reasonable cause for these problems, since a longer lifetime of refractory organic molecules in the reactor tube may allow the sample to fall down and become unevenly distributed on along the reactor wall. Users of this type of instrument have also noted a problem of tailing of the peaks on their CO_2 detection curves, which is consistent with this hypothesis.

The filtering step from TOC to DOC increased STD. Furthermore the analysis of DOC and TOC on the three freshwater samples and of the blank water revealed some contamination from filters. In five cases the reported DOC was higher than the TOC for the same sample. No significant differences were found between the different types or brands of the filters used by the participants. The laboratories reported limits of detection (LOD) in the range 0.05 - 2.0 mgC/L. There was however no consistency between the laboratories with low reported LOD and the lowest standard deviation. Since intercalibration containing a low OC concentration of NOM, was found to be significantly overestimated and showed a high relative standard deviation, it is therefore reason to be concerned that some reported LOD are too optimistic. Detection limit calculated according to the method described in Miller & Miller (2000) suggest 0.60mgC/L to be a more realistic LOD.

3.2 Article II: Artefacts in XAD-8 NOM fractionation.

The intention of the study presented in this article was to test the NOM XAD-8 fractionation method for three potential artefacts that may have influence on the interpretation of XAD-8 generated data and demonstrate the order of their influence. Three working hypothesis of the method was challenged though a serie of laboratory experiments using RO-isolates and natural samples:

- The concentration effect: The ratio of the hydrophobic acid fraction (HPOA) to hydrophilic fraction (HPI) is independent of the original DOC concentration.
- The chromatographic effect: The composition of the eluded HPI fraction will remain constant during the XAD-8 fractionation.
- Stability and artefact essay: HPI and HPOA represent stable chemical defined fractions. Neither chemical alteration during the fractionation procedure nor artefacts due to chromatographic methodology will establish a new HPI/HPO equilibrium after a removal of either of the fractions.

Experiment 1: The Effect of NOM concentration on the HPO/HPI cut-off.

In this experiment the RO-isolates Birkenes (fall) and Svartberget (spring) originating from the NOMiNiC project (Vogt et al. 2001) were prepared in concentration series covering the concentration range 0 – 40 mgC/L and XAD-8 fractionated in four replicates of each sample. These RO-isolates were picked to represent NOM of different character and the concentration span were designed to deliberately exceed the recommendations found in the original XAD-8 methodology (Leenheer & Huffman 1976, Leenheer 1981) in order to test the capacity of the XAD-8 material.

When the relative proportions of the XAD-8 fractions were compared at different concentrations, both RO-materials revealed a tendency of increase in the relative proportion of the HPI fraction and thereby an apparent higher “hydrophilic” character at higher concentrations. This increase in hydrophilic character continues over the whole concentration range, thus no clear limit at which the capacity of the XAD-8 material is exceeded was found. As a result the material appeared to be more “hydrophilic” at higher concentrations. For the total sample and the HPOA fractions no significant differences in sUVa and SAR were found between the different DOC concentration levels. In the HPI fractions, however, sUVa was found to increase significantly with the DOC concentration for both tested materials, and

SAR from the Svartberget material decreased significantly with concentration. These results indicate that when XAD-8 fractionation is performed at high DOC concentration, larger and more aromatic molecular structures are found to be present in the HPI fraction than found at lower concentrations. As a real increase in the hydrophilic character for a NOM material at higher concentration seems unlikely, a methodical bias seems sounder. If the amount of hydrophobic substance locally exceeds the capacity of the stationary phase then the NOM will leak faster through and out of the column at higher concentration. This would lead to a relatively larger HPI fraction consisting of larger and more aromatic NOM. This explanation is well supported by the sUVa and SAR observations. This result may have implications when the ratio hydrophobic to hydrophilic matter is compared for samples with different DOC concentration.

Experiment 2: Consistency of HPI during fractionation

In this experiment used samples from the same concentration serie prepared from the Svartberget RO-isolate described in the previous experiment. The samples were XAD-8 fractionated by the same method, but instead of collecting the HPI fraction as a whole, seven 20 mL aliquots were collected throughout the fractionation. Normalized 254 nm absorption (A_{254nm} HPI fraction/ A_{254nm} total sample) and specific absorption ratio (SAR) of these aliquots were then studied in order to detect changes in the HPI-fraction composition during the span of the fractionation.

For all samples the normalized 254 nm absorption of the HPI fraction increases significantly during the fractionation and only in the highest concentration approached a stabile level after about two thirds of the fractionation time. An increase in the 254 nm absorption could be caused by two factors separately or in combination; a) an increase in the overall NOM concentration of the aliquot, and/or b) an increase in the average sUVa of the NOM species in the aliquot. Regardless of cause, an increase in relative 254 nm absorption is a clear indication of instability in the composition of the HPI fraction during the fractionation. SAR decreased in the HPI throughout the fractionation for all concentrations but most profoundly in the samples with low NOM concentrations. These results are consistent with a constant increase in the leakage of more hydrophobic matter through the column due to the chromatographic nature of the method and the increasing saturation of the column with higher DOC concentrations. In the first aliquot of HPI there is a close to 50 % difference in the normalized 254 nm absorption between the lowest and highest concentration. Since XAD-8 fractions often are collected manually as a small sub samples during the procedure or when several columns are run en sequence, this artefact may have implications for the interpretation of the results. It will then be of major importance exactly when the sub-samples are collected. The results of these methods will therefore be highly operationally defined, and great care should be taken when performing the fractionation.

Experiment 3: Stability and artefact essay

In this experiment stored HPI and HPOA fractions from five previous XAD-8 fractionations were re-fractionated on XAD-8 columns. The stored fractions used in this experiment were from four natural surface water samples from Svartberget and Skjervatjern originating from the NOMiNiC project (Vogt et al. 2001) and a corresponding RO-isolate (Svartberget spring).

These original XAD-8 fractions had been pH adjusted back to the natural pH of the original samples (pH 4.5-5.5), and had at the time of the second fractionation been in storage in a dark cooling room for a period of 12 to 24 month (dark and at 4°C). Furthermore, in order to investigate the stability of HPI and HPOA fractions in the fractions on a shorter time frame, the 2nd HPI and HPOA fractions from the original XAD-8 fractionation were subjected to a third XAD-8 fractionation only 24-48 hours after the second. This time span is considered to represent the shortest and longest possible timeframe for any chemically or biologically induced changes to the fractions. The original fractionation was performed on a larger scale XAD-8 method (20 mL XAD-8 column and a sample volume 2 L), but the second and third re-fractionations were performed in 3 to 4 parallel sub-sample series on 3 mL XAD-8 columns to obtain standard deviation on the results. Individual HPI and HPOA fractions were pooled together to provide sufficient material for the third XAD-8 fractionation. The fractions were not filtrated prior to further XAD-8 fractionation, as normally required by the procedure in order not to disturb any larger NOM entities formed during the storage. TOC and 254, 400 and 600 nm absorbance were measured for all individual fractions.

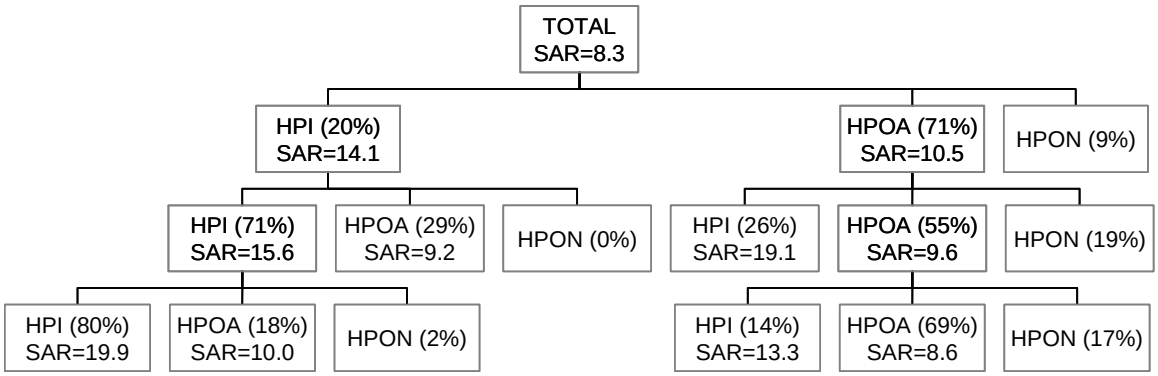

Figure 1: Fractionation scheme, relative distribution and SAR values of fractions from repeated XAD-8 fractionation of HPI and HPOA fractions (e.g. the Svartberget RO-isolate).

All samples revealed a pattern of "re-generation" of HPI and HPOA in the opposite fractions when subjected to the second XAD-8 fractionations and this observation is repeated in the third fractionation (figure 1). The boundaries between the two fractions seem therefore weak or altered through storage. Only minor differences in the fractionation/re-fractionation pattern could be found when natural samples from Svartberget were compared to the corresponding RO-isolate. This leads to the conclusion that the regeneration of HPI, HPOA and HPON is formed independently of the type of material (fresh sample or RO-isolate). Each time a sample was fractionated the HPOA fraction became generally more "hydrophobic" and the HPI fraction more "hydrophilic" of nature based on the total division into HPON, HPOA and HPI. The SAR values of the HPI fractions increased with each re-fractionation, indicating that the average molecular size of this fraction decreases with each fractionation. Similar, SAR of the HPOA-fractions were decreasing, indicating the presence of larger and more aromatic compounds in this fraction for each repeated fractionation.

These observations could be explained reproduction of HPI in the HPOA fraction and vice versa caused by irreversible changes in the chemical structures of the NOM material. The conditions of the XAD-8 procedure differ radically from the natural conditions of NOM and are very likely to cause permanent chemical alteration to the material. Extremely high pH is

found to cause a significant amount of breakdown due to hydrolysis in NOM material (Brinkmann et al. 2003). It is therefore very likely that the regenerated HPI found in the re-fractionation of HPOA could be created as a result of the desorption from the column with 1 N NaOH. The relatively high SAR values of the HPI fraction in the re-fractionated HPOA fractions support this theory. Another possible explanation could be that the cut-off boundary between the HPI and HPOA fractions is not so rigid. Due to the chromatographic nature of the XAD-8 procedure, the more hydrophobic compounds among the HPI fraction do no longer have to "compete" with the more hydrophobic compounds on the column. This corresponds quite well with the theory of capacity, saturation and chromatographic nature of the XAD-8 column. Finally the apparent generation of the HPOA in the HPI fractions and vice versa could also be explained by a chemical or biological induced change during storage, i.e. new equilibrium processes that aggregates or breaks down the NOM material. The observed alteration in the chemical structure of the fractions will not be significant as long as the purpose is to observe the mass ratio between the fractions. However, when the intentions are to analyze the fractions further in order to interpret their physicochemical properties, the user of the XAD-8 for NOM fractionation or isolation should be aware of this lack of stability.

3.3 Article III: The nature of the residual organic matter in drinking water following treatment with Chitosan and combined Chitosan / iron coagulants.

The main aim of this article was to investigate the physico-chemical characteristics of residual OM in raw water after treated with Chitosan and Chitosan/iron by means of different analytical methods expressing the hydrophobic character of the material. Furthermore, any structural changes in the residual OM upon storage were studied. XAD-8 fractionation and UV-, visual- and fluorescence spectroscopy have been used to elucidate the characteristics of the remaining OM, and the main question of analytical interest is how well these applied methods overlap in their conclusion on the hydrophobic nature of the OM. The purpose of this study has also been to document if the most successful NOM removal regime is achieved by using Chitosan treatment alone or in combination with metal coagulants.

The Chitosan applied in the study (Chito Clear TM 324, Primex, Norway/Iceland) was 94% deacetylated and had an average molecule weight of about 110 kD which is relatively large compared to most NOM. The Chitosan was dissolved in 0.5 weight % HCl (14 mM, pH ≈ 1.9). The iron chloride sulfate solution (JKL, Kemira Chemicals) had a 0.2 M Fe^{3+} concentration (11.6 weight %) and was applied undiluted. The stoichiometric ratio between chloride and sulphate in the solution was approx. 2:1. Raw-water was prepared by dissolution of a synthetic humic acid with 39% C content (sodium salts, Sigma-Aldrich Chemie GmbH) in 1 L tap water in an amount corresponding to a TOC level of 4.3 mgC/L. The organic matter content of the water was designed to reflect the humus rich waters often used as raw water source for the production of drinking water. Coagulation experiments were performed in 1-L beakers using "jar-test" units (Kemira Chemicals) with a programmable mixer, and the water was then filtrated through a Whatman GF/C membrane filter (1 μm pore size). Three different coagulant mixtures were tested: Low dosage of Chitosan (Treatment 1: Chitosan 1.75 mg/L), Optimum dosage of Chitosan (Treatment 2: Chitosan 5.0 mg/L) (Vogelsang et al. 2004), and a combination of 2.0mg/L Chitosan with 2.0 mg/L iron chloride/sulphate (Treatment 3).

Raw- and treated water samples were stored at 4°C in darkness. All samples were analysed by UV-VIS absorption, fluorescence and XAD-8 fractionation after four different storage periods; 1.5 hours, 1 day, 7 days, and 28 days.

Comparing the results from the XAD-8 fractionation of the original water with that of the three treatments show that the processed water have a significantly lower HPO/HPI ratio. This implies that the Chitosan mainly removes the hydrophobic matter. This observations are supported by the spectroscopic measurements: The low dosage treatment reduced sUVa to about 2/3 of the raw water while the two other treatments reduced sUVa to 1/3 of the original sample. SAR displays an increase with treatment. This *blue shift* in the spectra together with the reduced sUVA, suggest a loss of longer structures of conjugated double bonds and therefore be interpreted as a decrease in the more hydrophobic and larger structures of the NOM with the treatment. The total decrease in fluorescence is more or less proportional to the total removal of DOC by the three different treatments. Relative fluorescence spectra show only minor changes with the treatment. The humification index (HiX) decreased with increased amount of coagulants in the different treatments, which further supports that, a preferential precipitation of high-Mw structures.

The applied Chitosan dose in the two treatments involving Chitosan alone (Treatment 1 and 2) display a clear linear relationship (i.e. passing through origo) with the total NOM removal. When this relationship is compared with the effect of the combined treatment (3), it can be observed that the combined treatment is more than three times as efficient in total NOM removal than the dose of Chitosan alone in this treatment should account for. 2.0 mg/L iron is a relatively low dose compared with other studies (Vogelsang and Liltved 2001). A synergy effect between Chitosan and iron is a reasonable explanation since the two coagulants operate by somewhat different mechanisms: Coagulation with iron work primarily by charge neutralization and because of its smaller size it has access to a larger proportion of the NOM complexes than the polymer Chitosan. The coagulation of NOM with Chitosan is also to some extent based on charge neutralisation, but the large molecular size of the polymer, inhibit full access to the NOM complexes. On the other hand Chitosan offers the possibility of inter-particle bridging between NOM aggregates and thereby more efficient build up aggregates of a size that would no longer be stable in solution. Since it is of interest to keep the total addition of coagulants to a minimum, these results suggest that the combined treatment of Chitosan and iron is the most promising candidate for further studies.

While no significant changes were observed in any of the UV-VIS and fluorescence spectra over the storage period, the XAD-8 fractionations of the same samples revealed major changes in the distribution between hydrophobic and hydrophilic matter already during the 7-days period. No significant changes occurred among the XAD-8 fractions of the raw water sample. All the treated samples displayed increased hydrophobic character with time in storage, and thereby became more similar to the original raw water sample. The apparent discrepancy between the storage results on the optical properties and the XAD-8 fractionation could be due to that the changes in the structure of the humic matter does *not* involve changing the frequency and distribution of covalent conjunct double bonds (structure on the micro-scale), but rather the total molecular or aggregate size and charge density of the humic matter. The composition of XAD-8 fractions is likely to be in some sort of kinetically constrained equilibrium with each other and if one is removed e.g. by preferential precipitation, filtration or chromatographic processes, a “regeneration” of the lost fraction with time by a slow approach towards a new equilibrium between NOM in various degrees of

aggregation and colloidal state is therefore likely to occur as also observed in the XAD-8 study (article II).

Furthermore, if assumed that not all of the added flocculants actually precipitate and are removed by filtration, but that a minor fraction remains in the treated sample it would allow the remaining flocculants to continue to build aggregates after filtration. Such secondary aggregation with Chitosan would provide an increase in aggregate size and an elevated hydrophobic nature (i.e. as measured by XAD-8 fractionation) without leading to major structural changes in the covalent double bond structure of the humic matter. These results again pose questions to what is actually measured in the XAD-8 fractionation procedure. The method is un-doubtly linked to the hydrophilic/hydrophobic nature of the NOM, but maybe not the hydrophilic/hydrophobic character as it is normally defined in physical chemistry since the results of XAD-8 analyses is highly dependent on the outer conditions (matrix) surrounding the humic material. Combined with other information, the XAD-8 analysis can on the other hand provide information on the size and expressed hydrophilic character or perhaps average mobility of the NOM in the sample under the defined conditions.

3.4 Article IV: The effect of forest liming on humic mobilization and physico-chemical properties.

In this article the result of two major research projects are combined to provide information on the humic mobilization observed after the terrestrial liming of the Gjerstad experimental site:
The long term monitoring from the liming study on freshwater samples (Hindar et al. 2003, Hindar 2005) were combined with the multidimensional international collaboration study of the NOM-typing project (Gjessing et al.1999) involving RO-isolates obtained at the same sites with the aim to:

- Determine a mechanism behind the observed TOC mobilization.
- Determine the consequences for physio-chemical properties.

The Gjerstad experimental site was limed with 240 tons of coarse-grained (0-2 mm) dolomite [$CaMg(CO_3)_2$] in September 1994, corresponding to a dose of 2.9 t ha^{-1} (Hindar et al., 2003). The stream water in from the research and reference catchments was monitored and samples were collected every two weeks over the period from May 1993 to June 2002. All samples were analysed 2-3 days after sampling providing major cat- and anions are available along with pH, alkalinity, TOC and aluminum speciation. The NOM typing study included two RO-isolates from these catchments collected in May 1996, i.e. 20 months after the lime was applied. These RO-isolates have through the NOM-typing project been subjected to a large number of different analytical procedures in order to reveal their structure and physiochemical properties (Gjessing et al. 1999). Even though the uncertainty in comparing differences between these to RO-isolates are large, the whole set of isolates from the NOM-typing project could provid usefull information on the natural span found in the various parameters of humic materials. Thus the significance of the difference between the two Gjerstad Ro-isolates could be related to this natural span, and analytical parameters displaying large relative difference compared to the natural variation could be identified.

The TOC concentration of the streams at the two Gjerstad sites (limed and reference) are closely correlated with the precipitation intensity, displaying high TOC levels at high precipitation and low TOC during dry periods a pattern of discharge typically observed. Prior to liming a lower TOC concentration (about 30%) was measured in stream water draining the limed catchment relative to the runoff from the control site. During the eight years following the liming the TOC concentration in the runoff from the limed site increased significantly relative to the control site leading to similar TOC levels in the streams in the autumn of 2002. The increase in average TOC concentration in the runoff from the limed site equals about 0.25 mg C L^{-1} y^{-1}, corresponding to 2 mg L^{-1} over the whole period (significance 95 %), compared to a smaller and insignificant increase on the reference side. A general increase in surface water TOC and especially colour has been observed in northern Scandinavia during the last decades (Nordtest 2003). Still accounting for this general increase in background colour, at least 2/3 of the increase in TOC observed in the limed Gjerstad site can be assigned to the liming.

Several factors could explain an increase in TOC following as an effect of the terrestrial liming: a) increased terrestrial production, b) increased micro-biological degradation or c) mobilization of less soluble terrestrial NOM. No change in forest production was observed during the observation period following the liming (Hindar et al., 2003), thus this hypothesis is rejected as this effect probably would need a longer time span and can not explain the rapid change in TOC chemistry observed in the Gjerstad catchment. The second and faster mechanism to explain the increase in TOC could be increased micro-biological degradation of organic matter due to higher pH in the soil. Such an increase in micro-biological turnover of organic matter has been reported in the literature as a possible consequence of liming and increased pH (Mathur & Farnham 1985), but this hypothesis is difficult to confirm or reject from the available dataset. A third possibility would be increased mobility of TOC already present in the soil matrix. TOC solubility and thus mobilization is highly dependent on its charge density, which in turn is dependent on pH. The pH in the surface water of the experimental site changed from the initial level of 4.5-5.0 to a stable level around pH 6 after liming. Since a fraction of the weak organic acids of the NOM material have pK_a values in this range, a higher level of free charge per mgC is expected. Free organic charge (FC) has been modelled by subtracting an estimate of the bicarbonate from the acid charge balance and divided by TOC concentration (Equation 2). An estimate of the bicarbonate concentration was calculated from the alkalinity of the samples. The alkalinity was adjusted for the amount of H^+ consumed by the auto-protolysis of water. This model provides a relatively conservative estimate that is likely to underestimate the average organic charge slightly since some organic acids are protonized during the alkalinity titration.

Equation 2: Free charge per mgC modelled from charge balance (μeq mgC^{-1})

$$FC_1 = \frac{[H^+] + [K^+] + [Na^+] + [NH_4^+] + 2[Ca^{2+}] + 2[Mg^{2+}] - [Cl^-] - [NO_3^-] - 2[SO_4^{2-}] - [HCO_3^-]}{[TOC]}$$

The model displays a clear increase (approximately 25-30% μeq mgC^{-1}.) in average organic charge after the liming of the experimental catchment. This increase in free charge is so large that it alone probably could explain the mobilization of the TOC observed in the treated catchment.

Also the two RO-isolates from the streams draining the limed and control catchments, sampled two years after the liming, show some noteworthy differences. The proton binding were significantly higher on the limed- compared to the control site. When looking at the lowest pK_a interval (4.0-4.9) the increase in binding capacity is about 20%, the second pK_a interval (6.5-7.4) the limed side is 50% higher, and at the highest pK_a interval the capacity of the RO-isolate from the limed catchment is more than twice the capacity found at the reference site. These differences fits with the suggested liming-induced mobilization; TOC with a high content of medium to high pK_a valued acids would be mobilized as they are de-protonized by the increasing pH, while the effect on the lowest pK_a acids are minor importance since these acids already are active at pH 4.5.

The aromatic compounds increases when measured as an increase in sUVa, ratio of aromatic to aliphatic carbon measured by ^{13}C NMR and a slightly higher SAR. Increased aromaticity is in correspondence with the hypothesis of increased mobilization due to higher charge density since the otherwise lesser soluble aromatic TOC could gain increased solubility at higher pH. The most significant difference is found for the bioavailability measured by the octanol/water partition coefficient (K_{ow}) which is about 30 times higher on the limed side. This could be again be explained by the higher aromatic content together with the higher average pK_a values of the RO-isolate from the limed site. Only minor differences in the elemental content of the two RO-isolates, except for the sulphur content which is only about 1/10 in the sample from the limed site compared with the reference. Without further investigation it would be difficult to conclude if this is a result of the liming, different site characteristics or simply coincidence. The sulphate in the runoff decreased in both sites over the observed period with the sulphate concentration of the limed site been slightly higher over the total period and the mechanism here might have been desorption of sea-salt derived SO_4^{2-} in the soil (Hindar, 2005)..

The total stream concentration of organic nitrogen increased slightly in the limed catchment compared with the reference catchment. This increase does, however, not keep up with the increase in TOC, which results in a slightly decreasing trend in the relative organic nitrogen content of the NOM in the limed catchment, which could be a result of mobilization of older and more humified material. With no increase in NO_3-N and a significant decrease in NH_4-N in the stream water, there are no signs of nitrogen leakage as a result of increased bacterial degradation. This does, however, not exclude that micro biological activity has increased as a result of the liming, since the moderate dose of dolomite applied in the Gjerstad experiment may stimulate a more slow increase in the decomposition of the organic matter, in which a slower release of nitrogen species would have time to be consumed and recycled directly by vegetation and micro organisms.

4. Summary of conclusions

The intercalibration study on the *NOM high-temperature catalytic combustion* method revealed that a number of the participating laboratories were clearly too optimistic regarding their own precision, accuracy and detection level. This is likely to be the result of using mainly lighter and more combustible standard materials for instrumental monitoring of analytical performance since the accuracy and precision were high for the typical readily oxidizable standard material, but decreased for natural aquatic samples containing less readily oxidizable natural organic matter. High NOM concentration and a high level of recalcitrant organic matter proved to be challenge to combust completely and was more likely to be underestimated, especially the case for instruments with horizontal reaction tube. Higher reactor temperature did not influence significantly on the results suggesting that other factors than reactor temperature is limiting on the analytical performance of the tested instruments and laboratories. Statistical computation of limit of detection (LOD) suggested 0.60mg C L^{-1} to be a realistic detection limit based on potassium hydrogen phthalate, but this may be too optimistic if applied to analysis of not readily oxidizable natural organic matter. Cu-phthalocyanine-tetrasulfonic acid proved to be the lest readily oxidizable of all the samples and the analytical performance for samples based on natural organic material (i.e. fresh water and RO-isolates) were found to be in between these two extremes. Laboratories analysing natural organic matter by high-temperature catalytic combustion instruments are therefore encouraged to document and monitor their detection limit, accuracy and precision using standard natural material like RO-isolates or natural NOM material on a regular basis. The intercalibration study confirmed the need for standard analytical procedures for the analysis of NOM on high-temperature catalytic combustion instruments.

The XAD-8 fractionation study confirmed the suspicion that this NOM fractionation method is of a highly sample dependent and operational nature to a degree that may restrict the interpretation of the results or the ability to compare studies if these effects are not considered. The method revealed to have no well defined cut-off between fractions operational defined as hydrophobic and hydrophilic. The cut-off proved to be depending on the concentration of the sample and the composition of the hydrophilic fraction shifted towards larger and more aromatic NOM during the fractionation procedure. These effects is likely to be the results of the highly chromatographic nature of the XAD-8 fractionation procedure in combination with the capacity and saturation of NOM on the XAD-8 column and not safe and reliable concentration range could be found for the tested materials. The study further revealed that the physiochemical properties and structure of the individual species of the HPI and HPOA fractions are irreversibly altered due to the fractionation procedure and/or storage in a high ionic strength matrix as HPI material may be produced in the HPOA fraction and vice versa. This could raise questions regarding the use of XAD-8 generated fractions or samples for structural discussions and may touch upon the very understanding and discussion of hydrophobic/hydrophilic nature as an independent physiochemical feature of the NOM. The main conclusion from this study is not that studies based on XAD-8 fractionation are not useful, but with the literature study on the current scientific use of XAD-8 fractionation in mind, researchers using this method in NOM research are encouraged to keep in mind the highly operationally defined nature of these methods and be aware of the discussed analytical artefacts, when they plan and conduct their experiments and ultimately document their method and discuss results in publications.

The OC Chitosan complexation study demonstrated how several analytical parameters interpreted in combination can reveal a more complex picture of the structural and physiochemical properties of the NOM material. The coagulants initially displayed a clear preference toward removing the more hydrophobic matter as measured by both optical properties and XAD-8-frationation. When stored, however, the samples treated with Chitosan or a combination of Chitosan and iron displayed major changes in the ratio between hydrophobic and hydrophilic matter measured by XAD-8 fractionation. During several weeks of storage the hydrophobic/hydrophilic ratio became more and more similar to the original water. No changes were observed in the optical properties of any of the samples. This is very consistent with the results of the XAD-8 study indicating that XAD-8 fractionated samples may change and reorganise during storage. The results suggest that even though the interpretation of optical measurements and XAD-8 fractionation overlap to a large extent, they may represent physiochemical information at different structural levels of the OC. The potential consequences of this structural information in the case of Chitosan as a useful tool in the removal of OC from drinking water are not investigated in this study. Questions to bear in mind after this study are to what degree these structural changes on various levels are beneficial or not in water cleaning technology, whether Chitosan precipitates completely and if not potential consequences, how any traces of Chitosan can be measured in the processed water, and whether aquatic NOM as found in natural water supply locations behave in the same way as suggested in this study using an artificial humic standard.

The study on terrestrial liming proved to be a good example of how large collaboration multidimensional studies of physiochemical properties on standard NOM material can be combined with more classical long term field monitory projects with great benefit. Both approaches had their strong points and proved valuable supplements to each other as they together offered a greater insight in the behaviour of NOM in a limed catchment, than each of the studies could supply alone. The study concludes that NOM was mobilised after whole-catchment forest liming and that this increase in stream-water NOM can to a large extent be explained by increased mobility due to a clear increase in average organic charge. The mobilized NOM had a significantly higher total concentration of acidic sites and that a higher content of sites had high pKa values compared to the un-limed catchment. This offers not only a confirmation on the mobilization of NOM as a result of the liming, but also offers a mechanistic explanation to the mobilization. As a secondary consequence of the increased mobility due to increased free charge, indications of a higher fraction of aromatic compounds were also found in the lime treated NOM material. The relative content of organic nitrogen decreased slightly after the liming which could indicate that older and more humified material was mobilized.

As a overall closing remark on these studies it could be concluded that even though the study of NOM faces great analytical challenges from the very use of standard terminology and basic instrumental measurements to the theoretical deduction of NOM properties from methods and ultimately the consequences for the NOM function in the environment, possible solutions are offered in terms of collaborative intercalibration, method development, and the resent availability of new NOM standard materials from multidimensional studies of NOM functionality.

5. Further research

The research of humic substances will continue to be a battle against uncertainty deriving from the huge temporal and spatial variations observed in nature and the complexity in structure, functionality and composition found in any sample containing natural organic matter. In addition to these factors, the research field of humic matter is young, the potential scientific problems are manifold and the humic research community in rapid development.

Even though the ultimate reason for conducting humic matter research is to understand the behavior and characteristics of NOM in the natural environment, the analytical challenges behind this research should not be neglected even for highly popular methods. Given the scientific complexity and total publication volume in this research field, it can probably be concluded that discussion on analytical challenges, limitations and artifacts get too little attention in comparison with the total research field. More critical articles covering the use, understanding and standardization of methods should be published. Several of the results found in the research of this thesis, suggest overconfidence in established methods. Especially the link between NOM concentration and analytical performance, and the indication of reorganization and aggregation in manipulated NOM samples need further research. Where do we draw the boundary between molecular size and humic matter colloid? Is humic matter of a more dynamic and concentration dependent structure? And what potential consequences does this have for the interpretation of humic matter functionality?

The concepts of NOM size and aggregation, hydrophobic/hydrophilic nature and mobility need to be challenged and discussed further. What is for example conceptually understood by the term "hydrophobic" in the context of humic matter? Is it a property primarily deriving from characteristics of the humic molecule itself independently of its surroundings, as would be a more physiochemical content of the term hydrophobic? Or is it an attempt to describe the expressed hydrophobic *behavior* of the humic substance taking the impact of the surrounding matrix; NOM concentration, pH, ionic strength and available surfaces into account? In that case what is the purpose of the study? To reveal information on humic matter mobility, bio-availability or structure? And, finally, how can analytical procedures provide this information and what are the limitations and potential artifacts of these methods?

In the articles of this thesis it was demonstrated on multiple occasions the great benefit of the availability of stabile, standard NOM materials like the RO-isolates for a multitude of purposes covering everything from analytical merit documentation and method development to the ultimate analysis of NOM properties in nature. Large multidimensional collaborative studies like the NOM-typing and NOMiNiC project also proved to be of high value in the investigation and understanding of causes behind the natural variation in NOM structure and functionality found in the natural environment, and the effort to further collect and classify standard humic materials through projects like these should be continued.

There will always exist a dynamic relationship between purely analytical development and the actual study of NOM in the environment: Method development leads to a better understanding of NOM structure and functionality, which in turn may lead to new revision and improvement in the analytical schemes. Even though method development and critical reviews seem too time-consuming and at times also discouraging, they remove unnecessary errors and help toward a better and safer interpretation of the nature and behavior of humic matter.

References

Abbt-Braun, G.; and Frimmel, F.H. (1999). Basic characterization of Norwegian NOM samples – Similarities and differences, Envir. Int. 25: pp. 161-180.

Achan, O. (1908). Soluble humus material of northern fresh waters. Journal fuer Praktische Chemie 77: pp. 172-189.

Aiken, G.R.(1985). Isolation and Concentration Techniques for Aquatic Humic Substances. In Aiken, G.R.; McKnight, D.M.; Wershaw, R.L.; and MacCarthy, P. (eds). Humic Substances in Soil, Sediment and Water. Geochemistry, Isolation and Characterization. John Wiley and Sons, New York. ISBN 0-471-88274-7: pp. 363-385.

Aiken, G.R. (1988). A critical evaluation of the use of macroporous resins for the isolation of aquatic humic substances. Life Sci. Res. Rep. 41 (Humic Substances and Their Role in the Environment): pp. 15-28.

Aiken, G.R.; McKnight, D.M.; Thorne, K.A.; and Thurman, E.M. (1992): "Isolation of hydrophobic organic acids from water using nonionic macroporous resins", Org. Geochem. 18: pp. 567-573.

Andersen, D.O.; Alberts, J.J.; and Takács, M. (2000). Nature of Natural Organic Matter (NOM) in Acidified and Limed Waters, Wat. Res. 34: pp. 266-278.

Backlund, P.; Wondergem, E.; Voogd, K.; & de Jong, A. (1989). Mutagenic activity and presence of the strong mutagen 3-chloro-4-(dichloromethyl)-5-hydroxy-2-(5H)-furanone (MX) in chlorinated raw and drinking waters in The Netherlands. Sci. Tot. Envir. 84: pp. 273-282.

Benner, R.; and Strom, M. (1993). A critical Evaluation of the analytical blank associated with DOC measurements by high-temperature catalytic-oxidation. Mar. Chem. 41(1-3): pp. 153-160.

Bergström, I.; Mäkelä, K.; and Starr, M. (1995). Integrated Monitoring Programme in Finland. First National Report. - Ministry of the Environment, Environment Policy Department, Helsinki. Report 1, 138.

Bergström, I. (1998). The Integrated Monitoring Programme in Finland. Boreal Envir. Res. 3(3): 201-203.

Bishop, K.; Petterson, C.; Allard, B.; and Lee, Y.H. (1994). Identification of the riparian sources of aquatic dissolved organic carbon. Envir. Int. 20: pp. 11-19.

Brinkmann, T.; Abbt-Braun, G.; and Frimmel, F.H. (2003). Alkaline Degradation of Dissolved Organic Matter Acta Hydrochim. Hydrobiol. 31: pp. 213-224.

Fessenden, R.J.; and Fessenden, J.S. (1978). The basics of Organic Chemistry, Second edition, Allyn and Bacon Inc., Boston, US.

Gjessing, E.T. (1992). The HUMEX project – Experimental acidification of a catchment and its humic lake. Envir. Int. 18(6): pp. 535-543.

Gjessing, E.T. (1994). HUMEX (Humic Lake Acidification Experiment): Chemistry, hydrology, and meteorogy. Envir. Int. 20(3): pp. 267-276.

Gjessing, E.T.; Egeberg, P.K.; and Håkedal, J. (1999). Natural organic matter in drinking water – The "NOM typing project", background and basic characteristics of original water samples and NOM isolates. Envir. Int. 25(2/3): pp. 145-159.

Grip, H.; Bishop, K. (1990). Chemical dynamics of an acid stream rich in dissolved organics. In: Mason, B.J. (Ed.). The Surface Water Acidification Programme. Cambridge University Press: pp. 75-84.

Guetzloff, T.F.; and Rice, J.A. (1994): Does humic acid form a micelle?, Sci. Tot. Environ. 152: pp. 31-35.

Harvey, G.R.; Boran, D.A.; Chesal, L.A.; and Tokar, J.M. (1983). The structure of marine fulvic and humic acids. Mar. Chem. 12: pp. 119-132.

Harvey, G.R.; and Boran, D.A. (1985). In: Geochemistry of Humic Substances in Seawater. Aiken, G.R.; McKnight, D.M.; Wershaw, R.L.; and MacCarthy, P. (eds). Humic Substances in Soil, Sediment and Water. Geochemistry, Isolation and Characterization. John Wiley and Sons, New York. ISBN 0-471-88274-7: pp. 233-247.

Henriksen, A.; Fjeld, E.; and Hesthagen, T. (1999). Critical load exceedance and damage to fish populations. Ambio 28: pp. 583-586.

Hesthagen, T.; Sevaldrud, I.; and Berger, H.M. (1999). Assessment of damage to fish populations in Norwegian lakes due to acidification. Ambio 28: pp.12-17.

Hindar, A.; Wright, R.F.; Nilsen, P.; Larssen, T.; and Høgberget, R. (2003). Effects on stream water chemistry and forest vitality after whole-catchment application of dolomite to a forest ecosystem in southern Norway. For. Ecol. Manage. 180: pp. 509-525.

Hindar, A. (2005). Whole-catchment application of dolomite to mitigate episodic acidification of streams induced by sea-salt deposition. Sci. Tot. Envir. 343: pp. 35-49.

IHSS (2005). International Humic Substance Society, Accessed June 28th 2005; <www.ihss.gatech.edu/ >

ISO (1999). Water quality -- Guidelines for the determination of total organic carbon (TOC) and dissolved organic carbon (DOC). International organization for standardization, Geneva, Switzerland. ISO 8245.

Jones, R.I. (1992). In: Salonen, K.; Kairesalo, T.; and Jones, R.I. (eds.). Dissolved Organic Matter in Lacustrine Ecosystems: Energy Source and System Regulators. Kluwer Academic Publishers, Dordrecht / Boston / London. ISBN 0-7923-1652-5:
pp. 73-91.

Koprivnjak, J-F.; Blanchette, J.G.; Bourbonniere, R.A.; Clair, T.A.; Heyes, A.; Lum, K.R.; McCrea, R.; and Moore, T.A. (1995). The underestimation of concentrations of dissolved organic carbon in freshwaters, Water Res. 29(1): pp. 91-94.

Leenheer, J.A.; and Huffman, E.W.D. (1976). Classification of organic solutes in water by using macroreticular resins, J. Res. US Geol. Surv. 4(6): pp. 737-751.

Leenheer, J.A. (1981). Comprehensive Approach to Preparative Isolation and Fractionation of Dissolved Organic Carbon from Natural Waters and Wastewaters, Envir. Sci. & Tech. 15: pp. 578-587.

Leenheer JA, Noyes TI (1984): "A Filtration and Column-Adsorption System for Onsite Concentration and Fractionation of Organic Substances from Large Volumes of Water", USGS Survey Water-Supply Paper 2230, United States Government Printing Office, Washington, pp. 1-16.

Leenheer, J.A. (1985). In: Aiken, G.R.; McKnight, D.M.; Wershaw, R.L.; and MacCarthy, P. (eds). Humic Substances in Soil, Sediment and Water. Geochemistry, Isolation and Characterization. John Wiley and Sons, New York. ISBN 0-471-88274-7: pp. 409-429.

Malcolm, R.L. 1989 Spectroscopic Approaches. In: Heyes MHB, MacCarty P, Malcolm RL, Swift RS, editors: Humic Substances II. In search of structure. John Wiley & Sons, Chichester, 303-324. ISBN 0-471-92279-X.

Mathur, S.P.; and Farnham, R.S. (1985). Geochemistry of Humic Substances in Natural and Cultivated Peatlands. In: Aiken, G.R.; McKnight, D.M.; Wershaw, R.L.; MacCarthy, P. (Eds.). Humic substances in Soil, Sediment and Water, John Wiley & Sons Inc., ISBN 0-471-88274-7: pp. 53-85.

Miano, T.M.; Piccolo, A.; Celano, G.; and Senesi, N. (1992). Infrared and fluorescence spectroscopy of glyphosate-humic acid complexes. Sci. Tot. Envir. 123/124: pp. 83-92.

Miller, J.C.; and Miller, J.N. (2000). Statistics for analytical chemistry, Fourth Edition, John Wiley & sons. ISBN 0-130-22888-5.

Millipore Corporate Headquarters, 290 Concord Rd., Billerica, MA 01821, USA, www.millipore.com (accessed June 30th 2005).

Moore, S.G.; Calabrese, E.J.; DiNardi, S.R.; & Tuthill, R.W. (1978). Potential health effects of chlorine dioxide as a disinfectant in potable water suppliers, Med. Hypoth. 4(5): pp. 481-496.

Mulder, J.; Matzner, E.; Gallardo, J.F.; and Tipping, E.W. (1999). PROTOS - Annual report for the third year, Commission of the European Communities, Brussels. Rapport 4/99:36.

Nordtest (2003). Increase in colour and amount of organic matter in surface waters. Position paper 009. Available at: www.nordicinnovation.net/_img/position_paper_9.pdf (accessed June 30th 2005)

Oden, S. (1919). The humic acids, studies in their chemistry, physics, and soil science. Kolloidchemische Beihefte 11: pp. 75-260.

Pempkowiak, J. (2005). NMR spectra of the NOM typing project, unpublished data (pers.com.), Institute of Oceanology, Sopot, Poland.

Perdue, E.M.; and Gjessing, E.T. (eds.) (1990), Organic Acids in Aquatic Ecosystems, John Wiley & Sons, New York. ISBN 0-471-92631-0.

Poole, C.F. (2003). The Essence of Chromatography. Elsevier, Amsterdam. ISBN 0-444-50199-1.

Rook, J.J. (1976). Haloforms in drinkingwater. J. Am. Water Works Ass. 68(3): pp. 168-172.

Senesi, N. (1990). Molecular and quantitative aspects of the chemistry of fulvic acid and its interactions with metal ions and organic chemicals. Part II - The fluorescence spectroscopy approach. Anal. Chim. Acta. 232: pp. 77-106.

Senesi, N.; Miano, T.; Provenzano, M.; and Brunetti, G. (1991). Characterization, differentiation, and classification of humic substances by fluorescence spectroscopy. Soil Sci. 152(4): pp. 259-271.

Serkiz, S.M.; and Perdue, E.M. (1990). Isolation of dissolved organic matter from Suwannee River using reverse osmosis. Water Res. 24: pp. 911-916.

Sharp, J. (1993). The Dissolved Organic Carbon Controversy: An Update. Oceanogr. 6(2): pp. 45-50.

Sharp, J.H.; Benner, R.; Bennett, L.; Carlson, C.A.; Fitzwater, S.E.; Peltzer, E.T.; and Tupas, L.M. (1995). Analyses of dissolved organic carbon in seawater: the JGOFS EqPac method comparison. Mar. Chem. 48: pp. 91-108.

Sharp, J. (1997). Marine dissolved carbon: Are the older values correct? Mar. Chem. 56: pp. 265-277.

Sharp, J.H.; Carlson, C.A.; Peltzer, E.T.; Castle-Ward, D.M.; Savidge, K.B.; and Rinker, K.R. (2002). Final dissolved organic carbon broad community intercalibration and preliminary use of DOC reference materials. Mar. Chem. 77(4): pp. 239-253.

Shimadzu 2000. Instruction manual, Total Organic Carbon Analyzer, Model TOC-5000A, Shimadzu Corporation, Tokyo, Japan.

Shinozuka, N.; Lee, C. (1991). Aggregate formation of humic acids from marine sediments, Marine Chem. 33: pp. 229-241.

Skoog, D.A.; West, D.M.; and Holler, J.F. (1988). Analytical Chemistry. Sauders College Publishing, New York. ISBN 0-03-14828-6.

Skoog, D.A.; and Leary, J.J. (1992). Principles of Instrumental Analysis. Sauders College Publishing, New York. ISBN 0-03-023343-7.

Skoog, A.; Thomas, D.; Lara, R.; and Richter, K.U. (1997). Methodological investigations on DOC determinations by the HTCO method. Mar. Chem. 56: pp. 39-44.

Steinberg, C.; and Muenster, U. (1985). Geochemistry and Ecologigcal Role of Humic Substances in Lakewater. In: Aiken, G.R.; McKnight, D.M.; Wershaw, R.L.; and MacCarthy, P. (eds). Humic Substances in Soil, Sediment and Water. Geochemistry, Isolation and Characterization. John Wiley and Sons, New York. ISBN 0-471-88274-7: pp. 105-145

Steinberg, C.E.W. (2003): Ecology of Humic Substances in Freshwaters. Springer-Verlag, Berlin. ISBN 3-540-43922-6.

Stumm, J.H; and Morgan, J.J. (1981). Aquatic Chemistry. Wiley Interscience, New York. ISBN 0-471-09173-1.

Stevenson, F.J. (1985). Geochemistry of Soil Humic Substances. In: Aiken, G.R.; McKnight, D.M.; Wershaw, R.L.; and MacCarthy, P. (eds). Humic Substances in Soil, Sediment and Water. Geochemistry, Isolation and Characterization. John Wiley and Sons, New York. ISBN 0-471-88274-7: pp. 13-52.

Tait, R.V. (1981). Elements of Marine Ecology, Butterworths, The University Press, Cambridge, ISBN 0-408-71054-3.

Takács, M.; Alberts, J.J.; and Egeberg, P.K. (1999). Characterization of natural organic matter from eight Norwegian surface waters: Proton and copper binding. Environ. Int. 25(2/3): pp. 315-323.

Thomsen, M.; Lassen, P.; Dobel, S.; Hansen, P.E.; Carlsen, L; and Mogensen, B.B. (2000). Characterisation of humic materials of different origin: A multivariate approach for quantifying the latent properties of dissolved organic matter, Chemosphere 49: pp. 1327-1337.

Thurman, E.M.; and Malcolm, R.L. (1981): "Preparative isolation of aquatic humic substances". Envir. Sci. Technol. 15: pp. 463-466.

Thurman, E.M. (1985). Organic Geochemistry of Natural Waters. Martinius Nijhoff & Dr W. Junk Publishers. Boston. ISBN 90-247-3143-7.

Tombácz, E. (1999). Colloidal properties of humic acids and spontaneous changes of their colloidal state under variable solution condition. Soil Sci. 164: pp. 814-824.

Tranvik, L.J. (1992): Allochtonous dissolved organic matter as an energy source for pelagic bacteria and the concept of the microbial loop. In: Salonen, K.; Kairesalo, T.; and Jones, R.I. (eds.). Dissolved Organic Matter in Lacustrine Ecosystems: Energy Source and System Regulators. Kluwer Academic Publishers, Dordrecht / Boston / London. ISBN 0-7923-1652-5: pp. 107-114.

Tsutsuki, K.; Kuwatsuka, S. (1979). Chemical studies on soil humic acids. VII pH-Dependent nature of the ultraviolet and visible absorption spectra of humic acids. Soil Sci. Plant Nutr. 25(3): pp. 373-84.

Vanoon, G.W.; and Duffy, S.J. (2002). Environmental Chemistry – a global perspective. Oxford University Press. Oxford. ISBN 0-19-85644-6.

Vogelsang, C.; and Liltved, H. (2001). "The use of Chitosan for removal of humic substances at Årnes water treatment plant A/L – the results from jar-tests" (in Norwegian), Norwegian Institute for Water Research (NIVA), Report LNR 4390-2001, Oslo, Norway.

Vogelsang, C.; Andersen, D. O.; Hey, A.; Hakonsen, T.; Jantsch, T. G.; Muller, E. D.; Pedersen, M. A.; Varum, K. M. (2004). Removal of humic substances by chitosan. Water Science & Technology: Water Supply 4(5/6): pp. 121-129.

Vogt, R.D; Andersen, D.O.; Bishop, K.; Clarke, N.; Gadmar, T.C.; Gjessing, E.; Lundstrøm, U.; and Starr, M. (2001), Natural organic matter in the Nordic Countries (NOMiNiC). NORDTEST report. Nordisk InnovationsCenter, Oslo, Norway. Available at: www.nordicinnovation.net/nordtestfiler/tec479.pdf (Accessed June 28th 2005).

Wershaw, R.L. (1999). Molecular aggregation of humic substances, Soil Sci. 164: pp. 803-813.

Williams, D.H.; and Flemming, I. (1980). Spectroscopic methods in organic chemistry, Third edition, McGraw Hill Book Company (UK) Limited, London, UK. ISBN : 0-07-709147-7.

Youden, W.J. (1959). Graphical Diagnosis of Interlaboratory Test. Industrial Quality Control: pp. 15-24.

Youden, W.J.; and Steiner, E.H. (1975). Statistical Manual of the Association of Official Analytical Chemists, Statistical Techniques for Collaborative tests. Arlington.

Zsolnay, A.; Baigar, E.; Jimenez, M.; Steinweg, B.; and Saccomandi, F. (1999). Differentiating with fluorescence spectroscopy the sources of dissolved organic matter in soils subjected to drying. Chemosphere 38: pp. 45-50.

Appendix A: Standard operational procedure for NOM XAD-8 fractionation (3 mL column)

Tone C. Gadmar, University of Oslo, Department of Chemistry

1 Introduction

1.1 General introduction

This fractionation procedure for natural organic matter (NOM) by the use of an Amberlite XAD-8 column is founded on the work of Leenheer and Huffman (Leenheer & Huffman 1976, Leenheer 1981). There are now a large variety of modifications and clones from the original method of 1976, adapted to suit very different analytical purposes. The XAD-8 material has over the last few decades also become a popular tool for isolation of NOM from an aqueous sample (Thurman & Malcolm 1981). The method has been adopted by International Humic Substances Society (IHSS 2005) and the international standards and reference material of fulvic- and humic acids is based on isolation using XAD-8.

XAD-8 is widely used to fractionate NOM into a hydrophilic and hydrophobic fraction by simply pumping a given volume of water sample at a fixed pH through the resin: The material retained on the resin is defined as *hydrophobic*, while the NOM that passes though the column is defined *hydrophilic*. The concentration ratio between the fractions is used to express the hydrophobic character of the NOM. The XAD-8 technology provides a highly operationally defined chromatographic method of separation and investigation of the NOM material. The most important implication of this is that small differences in methodology may make comparison between data from various methods and operators difficult or even impossible, and significant limitations potential analytical artifacts are reported. Several publications address this problem in critical reviews of the fractionation and isolation techniques (Aiken 1985, Aiken 1988, Leenheer 1985, Gadmar et al. 2005). The user of the XAD-8 technique should therefore be familiar with these reviews and critique and be aware of the operational nature and limitations of the method.

1.2 Dimensions

In this procedure 180 mL sample is fractionated into a hydrophilic fraction (HPI) and a fraction of hydrophobic acids (HPOA), using a 3 mL column of XAD8 material.

The method is suitable for samples in the range 5-25 mgC/L. Lower DOC concentrations will give larger uncertainty and higher may saturate the XAD8 column and cause bleeding of hydrophobic material into the hydrophilic fraction. Some samples with higher DOC contents may therefore be diluted with carbon-free water (MilliQ water) down to the acceptable range. The user should be aware that the DOC concentration may influence on the cut-off between the hydrophobic and hydrophilic fraction.

2 Preparation and packing of the XAD8 column.

2.1 Solutions and materials

Carbon-free water:
MilliQ or distilled water. All dilutions are made with carbon-free water.

NaOH:
1.0 N NaOH: 40.0 g NaOH pellets + 1000 mL water, pH 14
0.1 N NaOH: 4.0 g NaOH pellets + 1000 mL water, pH 13

HCl:
Concentrated HCl: 37 %, 12.1 M
1.0 N HCl: 8.3 mL cons. HCl + 100 mL water, pH 0
0.1 N HCl: 8.3 mL cons. HCl + 1000 mL water, pH 1
0.01 N HCl: 830 µL cons. HCl + 1000 mL water, pH 2

Organic solvents:
Methanol
Acetonitrile
Diethyl ether

Column material:
XAD8: Fluka Chemical
Glass wool

2.2 Quality control on solutions

The DOC content of all prepared aqueous solutions and carbon-free water should be monitored by DOC measurements and/or absorbance at 254 nm.

2.3 Equipment

Soxlet equipment

Glass bottles and vials for storage of samples and fractions:

Brown glass bottles and vials for storage of samples, fractions and column material are baked at 500 ^{o}C prior to use to remove organic material.

Clear or brown glass vials for storage of small aliquots of samples and fractions. Prebaked at 500 ^{o}C prior to use.

Glass columns of 4-5 mL volumes

Peristaltic pump with adjustable flow-rate (1-5 mL/min)

2.4 Preparation of the XAD8 material

XAD-8 (Fluka Chemical): This is a non-ionic column material with high adsorption capacity and good eluation properties. XAD-8 contains acrylic esters bond together in a polymeric resin. The material is easily broken down during use, because of low degree of side chains and stabilizing bonds. This will cause the material to brake down over time and start bleeding DOC. To compensate for this, the column is washed prior to use and blind samples are regularly analyzed.

Prior to the packing of the column the XAD8 material is soaked in 1 N NaOH for 24 hours and then subjected to sequential soxlet extractions in methanol, acetonitrile and ether (24 hours in each solvent). The XAD8 material is then stored in methanol in brown glass bottles.

Glass wool is subjected to soxlet extraction in methanol for 24 hours prior to storage in methanol.

Table 1: Properties of the XAD8 material.

material	matrix	uptake of solvent pr.g	Pore diameter	Pore volume	Surface area
XAD-8	akrylic ester	1.31-1.36	25 nm	0.83 cm^3/g	140 m^2/g

2.5 Packing of the column

3 mL XAD8 is packed in glass columns filled with methanol. A plug of glass wool is applied in the top of the column over the XAD8 to avoid migration of the material during back flushing.

2.6 Washing of the column.

Prior to first run the XAD8 column is washed by flushing it sequential with 0.1 N NaOH, 0.1 N HCl and methanol at a flow speed of 5 mL/min. (20 minutes for each solution), and then rinsing for an hour by flushing with carbon-free water (same flow speed). 10-20 mL of the last carbon free water is collected ad kept as a control of column bleeding.

Prior to every later run the column is washed by back-flushing the column sequential with 0.1 N NaOH and 0.1 HCl at a flow speed of 2 mL/min (15 minutes each), and then rinsing with carbon free water for 30 minutes at the same flow speed. 10-20 mL of the last carbon free water is collected ad kept as a control of column bleeding.

3 Preparation of the sample

3.1 DOC from natural samples

Natural samples intended for XAD8 samples should be prepared in the field laboratory immediately after collection to avoid biota to change the composition of the DOC. About 250 mL raw-sample is required per fractionation, since part of the sample is spent during filtration and some is prepared for DOC measurements. Samples are prepared by filtering using a 0.45µm membrane filter. To avoid biological activity, 0.5 mL 70 ppm AgNO3 solution can be added pr. 1000 mL sample. The samples should be stored pre-baked brown glass bottles in a dark cooling-room until they are fractionated and analyzed.

3.2 DOC from RO-isolates

RO-isolates are dissolved in carbon-free water (Milli-Q) to the desired concentration at least 24 hours prior to fractionation. About 250 mL raw-sample is required per fractionation, since part of the sample is spent during filtration and some is prepared for DOC measurements. The samples are prepared by filtering using a 0.45µm membrane filter. Samples are stored in pre-baked brown glass bottles in a dark cooling-room until they are fractionated and analyzed.

3.3 Measurement of DOC and optical properties on the samples

About 20 mL of each sample should be kept for DOC analysis and measurement of optical properties of the total sample prior to the fractionation. DOC is measured by high temperature catalytic combustion. Optical properties are measured as absorption at 254, 400 and 600 nm. Since absorbance is dependent of pH, all the sub-samples should be pH adjusted to pH 2.0 (± 0.1) prior to measurement.

4 Fractionation

4.1 Wash of the column

Prior to every run the column should be washed accordingly to the procedure B in section 2.5. 10-20 mL of the last carbon free water is collected ad kept as a control of column bleeding. This sample could be analyzed for DOC and/or absorbance could be measured as a quick check on the columns condition.

4.2 True blinds

True blind samples consisting of carbon-free water should be treated and run as an ordinary sample frequently (recommended after every third sample). The XAD8 material should be changed and the column repacked after 10-20 samples, depending on the DOC content of the samples, bleeding from the column and the values of the true blinds. As a rule of thumb 10 samples with an average of 10 mgC/L or 20 samples of 5 mgC/L could be run on the column. That is IF the bleeding and the true blinds are still low. If they are not, the XAD8-material should be changed more frequently. Some samples contain a higher level of hydrophobic neutral material that will be difficult to rinse out in the washing procedure. This will exhaust the XAD8 material more rapidly and course increasing blinds and bleeding from the column.

4.3 Acidifying the sample

Just before fractionation, 180 mL sample is acidified to pH 2.0 (± 0.1) by addition of concentrated HCl (37 %). 200-400 µL concentrated HCl is usually required to bring pH down to 2.0. Note the natural pH, the acidified pH and the volume of acid added to the sample.

4.4 Separation of HPO from HPI by the XAD-8 column

The whole 180 mL sample is pumped through the XAD8 column at a flow-speed of one mL/minute. The hydrophobic fractions (HPO) are adsorbed by the XAD8 material and the hydrophilic fractions (HPI) pass through. The first 30 mL is passing the column is thrown away (dead-volume of the column-system). The rest of the HPI fraction is collected.

When the whole sample is sucked up, a 30 mL volume of carbon-free water at pH 2 is run through the column to be sure to run the whole volume of sample through the XAD8 column. To avoid dilution of the HPI fraction, HPI should not be collected when the portion of acidified carbon-free water is run though the column.

4.5 Eluation of the HPO-A from the XAD-8 column.

The hydrophobic acid fraction (HPOA) is eluated by back-flushing the column with 1 N NaOH solution at a flow-speed of one mL/minute. This will cause the mobilization of the acid fraction of the HPO and cause them to flow back and out of the column. 30 mL of eluated hydrophobic acids should be collected. This is more concentrated than the original sample and 150 mL carbon-free water is added to dilute it back to the original HPOA concentration of the sample.

4.6 Measurement of DOC and optical properties on the fractions

About 20 mL of each fraction should be kept for DOC analysis and measurement of optical properties. DOC is measured by high temperature catalytic combustion. Optical properties are measured as absorption at 254, 400 and 600 nm. Since absorbance is dependent of pH, all fractions should be pH adjusted to pH 2.0 (± 0.1) prior to measurement or to another fixed pH according the objectives of the experiment.

4.7 Using the collected fractions for further analysis.

After the measurement of DOC and optical properties, 100-150 mL of the collected fractions is left unused. These fractions could be used for other investigation of the material as long as the difference in pH and ionic strength between the total sample, HPI and HPOA fractions are kept in mind. To investigate the fractions under similar conditions, pH and ionic strength should be adjusted to the same level for all the fractions by adding HCl, NaOH and NaCl. pH, conductivity and added volume of solutions should be noted. The user of the method should also keep in mind that the NOM material fractionated by the use of the XAD-8 procedure has been subjected to a very tough chemical treatment and that it is likely to have undergone structural changes compared to the original sample. This is especially the case for the HPOA fraction eluated under high pH.

References:

Aiken, G.R.(1985). In Aiken, G.R.; McKnight, D.M.; Wershaw, R.L.; and MacCarthy, P. (Eds). Humic Substances in Soil, Sediment and Water. Geochemistry, Isolation and Characterization. John Wiley and Sons, New York. ISBN 0-471-88274-7: pp. 363-385.

Aiken, G.R. (1988). A critical evaluation of the use of macroporous resins for the isolation of aquatic humic substances. Life Sci. Res. Rep. 41 (Humic Substances and Their Role in the Environment): pp. 15-28.

Gadmar, T.C.; Vogt, R.D.; and Evje, L.. (2005). Artefacts in XAD-8 NOM fractionation. Int. J. Envir. Anal. Chem. 85(6): pp. 365-376.

Leenheer, J.A.; and Huffman, E.W.D. (1976). Classification of organic solutes in water by using macroreticular resins, J. Res. US Geol. Surv. 4(6): pp. 737-751.

Leenheer, J.A. (1981). Comprehensive Approach to Preparative Isolation and Fractionation of Dissolved Organic Carbon from Natural Waters and Wastewaters, Env. Sci. & Tech. 15: pp. 578-587.

Leenheer, J.A. (1985). In: In Aiken, G.R.; McKnight, D.M.; Wershaw, R.L.; and MacCarthy, P. (eds). Humic Substances in Soil, Sediment and Water. Geochemistry, Isolation and Characterization. John Wiley and Sons, New York. ISBN 0-471-88274-7: pp. 409-429.

Thurman, E.M.; and Malcolm, R.L. (1981): "Preparative isolation of aquatic humic substances". Envir. Sci. Technol. 15: pp. 463-466.

Paper I

Paper II

Paper III

The nature of the residual organic matter in drinking water following treatment with chitosan and combined chitosan / iron coagulants

Tone C. Gadmar and Magnus Christiansen
University of Oslo, Dept. of Chemistry, P.O.Box 1033, N-0315 Oslo, Norway

Corresponding author: Tone C. Gadmar,
University of Oslo, Dept. of Chemistry, P.O.Box 1033, N-0315 Oslo, Norway,
Phone: +47 228 55446, Fax: +47 228 55441, Email: t.c.gadmar@kjemi.uio.no

Abstract

The natural organic matter (NOM) that remains in solution after coagulation treatment is of concern for many water works due to its potential to serve as a source of energy for microbial growth and cause bio-fouling in the distribution systems, and because of the generation of harmful disinfection byproducts (DBP). Chitosan is a natural biopolymer, which appears to be well suited to remove humic substances from drinking water. In laboratory scale experiments three different Chitosan treatment regimes were tested and the characteristics of the residual organic matter evaluated by studying the optical properties and distribution between hydrophobic/hydrophilic matter (by means of XAD-8 fractionation). The three different coagulant systems removed from 15 to 50% of the organic matter from the raw water. Furthermore, the residual NOM differed compared to the raw material both with regard to specific UV adsorption (sUVa), specific absorption ratio (SAR) and HPI/HPO-ratio. The latter ratio changed also considerable during one month of storage of the three residual materials, whereas the optical properties remained constant.

Keywords: Drinking water, coagulation, chitosan, NOM, UV spectroscopy, sUVa, SAR, fluorescence, XAD-8.

Introduction

In the Nordic countries, surface water is often used for the production of drinking water. About half (w/w) of "the total matter" in many surface waters, is of organic nature. This natural organic matter (NOM), originating mainly from soil, has a characteristic brownish yellow colour. The coloured natural organic matter, as such, is not considered to be of any hygienic concern. However, there are five main arguments for why NOM should not be present in tap water.

- NOM have the ability to form strong complexes with inorganic and organic micro-pollutants (such as Hg, Cd, Cu, PAH, PCB). Thus elevated amount of NOM may also carry a high loading of micro pollutants into the tap water.
- NOM form disinfection byproducts that may have mutagenic or carcinogenic properties (Rook 1976, Moore et al. 1978, Backlund et al. 1989).
- Part of the NOM is an energy source for micro-organisms in the distribution system, producing bio-fouling and most probably allergens and possibly myco-toxins.
- The color of NOM may stain the fabrics during washing
- The color is considered an esthetical problem

A commonly used method for removal of NOM is direct filtration after flocculation and precipitation with metal-based coagulants. However, some water works have found it difficult to comply with the low permissible levels of residual metal coagulant (aluminium in

particular). Moreover, a high metal content in the produced sludge, leads to costly sludge disposal since it is considered to be a potentially harmful waste. The biopolymer Chitosan, produced from crustacean waste (i.e shrimp shells), has been introduced as a promising alternative or supplement to metal-containing coagulants. Furthermore, the sludge produced with Chitosan is found to be more biodegradable than metal derived sludge. Chitosan has previously been shown to be an efficient colour remover. Based on the study of Vogelsang et al. (2004) it is shown that the Chitosan treatment preferably removes the largest molecular weight organic matter measured by size exclusion chromatography. On the other hand it is found to leave a higher proportion of the medium molecular weight organic matter in the treated water than the metal-based coagulants (Vogelsang et al. 2004). Co-addition of Chitosan and iron chloride, however, has shown similar treatment results as the metal-based coagulants alone, but at a significantly lower total coagulant addition (Vogelsang et al. 2004). The coagulating effect of Chitosan on NOM is due to a combination of charge neutralisation and inter-particle bridging (Vogelsang et al. 2004). Due to the potential problems of residual NOM, as indicated above, a better characterisation of the properties and stability of the remaining organic matter (OM) after coagulation with Chitosan is needed.

The presented work report on the physico-chemical characteristics of residual OM in raw water after treated with Chitosan. Furthermore, the structural changes that apparently take place in the residual OM upon storage are studied. XAD-8 fractionation and UV-, visual- and fluorescence spectroscopy have been used to elucidate the characteristics of the remaining OM. The purpose of this study has also been to document if the most successful NOM removal regime is achieved by using Chitosan treatment alone or in combination with metal coagulants.

Material and methods

Coagulants
The Chitosan (Chito Clear TM 324, Primex, Norway/Iceland) was 94% deacetylated and had an average molecule weight of about 110 kD. The Chitosan was dissolved in 0.5 weight % HCl (14 mM, pH ≈ 1.9). The iron chloride sulphate solution (JKL, Kemira Chemicals) had a 0.2 M Fe^{3+} concentration (11.6 weight %) and was applied undiluted. The stoichiometric ratio between chloride and sulphate in the solution was approx. 2:1.

Experimental water
Raw-water was prepared by dissolution of a synthetic humic acid with 39% C content (sodium salts, Sigma-Aldrich Chemie GmbH) in 1 L tap water in an amount corresponding to a TOC level of 4.3 mgC/L. The organic matter content of the water was designed to reflect the humus rich waters often used as raw water source for the production of drinking water.

Jar tests
Coagulation experiments were performed in 1-L beakers using “jar-test” units (Kemira Chemicals) with a programmable mixer. The coagulants were added during rapid mixing (400 rpm) for 60 seconds, immediately followed by flocculation during slow stirring (30 rpm) for 10 minutes. The water was then filtrated through a Whatman GF/C membrane filter (1 µm pore size). Three different coagulant mixtures were tested: Low dosage of Chitosan (Treatment 1: Chitosan 1.75 mg/L), Optimum dosage of Chitosan (Treatment 2: Chitosan 5.0 mg/L) (Vogelsang et al. 2004), and a combination of 2.0mg/L Chitosan with 2.0 mg/L iron chlorid/sulphate (Treatment 3).

Sample storage
Raw- and treated water samples were stored at 4°C in darkness. All samples were analysed after four different storage periods; 1.5 hours, 1 day, 7 days, and 28 days.

UV-VIS spectroscopy
The chromophores in NOM that are expected to absorb light most strongly are π-conjugated systems, commonly found in complex aromatic compounds (William & Flemming, 1980). Increased length of conjunct double bonds is known to shift the absorbance towards longer wavelength due to increased resonance of the molecular structure (Fessenden & Fessenden, 1978). An increased *red shift* (i.e. reduced specific adsorption ratio (SAR = A_{254nm} / $A_{400\ nm}$) is therefore, in the context of humic substances, associated with more hydrophobic and larger molecular structures (Abbt-Braun & Frimmel 1999, Malcolm 1989, Thomsen et al. 2002, Andersen et al. 2000).

UV-visible absorbance scans between 190 and 1100 nm were performed on an Agilent 8453E photodiode array spectrophotometer with accuracy of ± 0.005 absorption units (A.U.). Sample measurements were blank-corrected by subtracting the spectrum of each sample with the spectrum of carbon free water (Milli-Q185, Millipore 2004). The absorbance at 254 nm (A_{254}) and 400 nm (A_{400}) are used to calculate the specific UV absorption (sUVa; $A_{254}/mgCL^{-1}$) and specific absorption ratio (SAR; A_{254nm}/A_{400nm}). Since the treated samples display a relatively low total absorbance at 400 nm, a 300 nm version of "SAR" were calculated for the whole 250-600 nm spectra ($SAR_{300} = A_{\lambda}/A_{300nm;\{\lambda\in 250\text{-}600nm\}}$) in order to verify this blue shift throughout the spectra using a more significant reference wavelength.

Fluorescence spectroscopy
Fluorescence measurements were performed on a Perkin-Elmer LS-50B scanning spectrophotometer operated in the emission scan mode at 22 ± 2°C using matched 10 mm quartz cuvettes. Emission spectra were recorded between 380 and 550 nm at an excitation wavelength λ_{ex} = 360 nm. The wavelength range of the emission spectra was chosen as dissolved aquatic NOM (DNOM) is known to exhibit an emission maximum in the region 410 - 490 nm (Senesi, 1990). An excitation wavelength of 360 nm seems to be favored in fluorescence emission studies of DNOM (Senesi 1990, Senesi et al. 1991, Miano et al. 1992). The emission spectra were corrected for inner-filtration effects by multiplication with the factor e^{A}, where A is the sample absorbance at the excitation wavelength (Zsolnay et al. 1999). The spectra were subsequently used to calculate the Humification Index (HiX) as given in equation 1. In this study HiX was calculated to determine whether or not a preferential removal of high-Mw structures had occurred upon the different treatments. A decrease in the HiX of the DNOM, corresponding to a s.k. blue-shift, would be indicative of such preferential precipitation. Relative fluorescence spectra is obtained by dividing the total fluorescence by the concentration of carbon (mgC/L) in the sample

Eq.1 $HiX = I_{465\text{-}540} / I_{390\text{-}465}$ (I = emission intensity at each wavelength)

XAD-8 fractionation

XAD-8 fractionations (Leenheer and Huffman 1979, Leenheer 1981) were performed on all four samples on day 0, 1, 7 and 28. 180 mL samples (pH 2.0±0.1) were fractionated on a column filled with Amberlite XAD-8 material (approx. length 8 cm and inner diameter 5 mm, 3 mL resin volume, flow 1 mL/min). At pH 2 the operationally defined *hydrophobic fraction* (HPO) was retained by the XAD-8 material, while the *hydrophilic fraction* (HPI) passed

through the column and was collected for further analysis. The *hydrophobic acid* fraction (HPOA) was eluted by back-flushing the column with 1 N NaOH (pH 13). The 30 mL eluted HPOA materials were diluted back to their original concentration in the samples by adding 150 mL carbon-free water. The *hydrophobic neutrals* (HPON), is defined as the fraction that remains on the XAD-8 column at pH 13 (i.e. HPON = Total - HPI - HPOA). Blind samples of carbon-free water (Milli-Q185, Millipore 2004) were frequently treated and run as an ordinary samples in between the ordinary samples. Total organic carbon (TOC) concentration and UV-VIS absorption (254, 400, 600 nm) were measured on all HPI- and HPOA fractions. The four fractions (i.e. HPO, HPOA, HPI and HPON) of this procedure are primarily defined by the experimental conditions, and should not be interpreted as clearly separable groups of compounds.

TOC analyses

The carbon concentrations were determined in replicate using a Shimadzu TOC 5000A analyser with an IR detector (agreement within 0.2 mgC/L) (Shimadzu 1998). Since the TOC concentration in the samples and NOM fractions were low (0.5 - 4.3 mgC/L), a standard curve of 10 concentrations (+ blank) covering the 0.2-6.0 mgC/L concentration range were designed and analyzed as ordinary samples to verify the 0.2 mgC/L standard deviation on the TOC measurements. The total TOC concentration of the samples and hydrophilic- (HPI) and hydrophobic acids fractions (HPOA) were measured by direct TOC measurements, and the standard deviation of these values are therefore 0.2 ppm, while the hydrophobic neutrals (HPON), total hydrophobic fraction (HPO) and the HPO/HPI ratios are computed from several TOC measurements and thus have a standard deviation calculated to 0.3 mgC/L.

Results and discussion

Table 1 gives a summary of the results from the XAD-8 fractionation, UV-VIS absorbance spectroscopy and TOC analyses

XAD-8 Characteristics

Comparing the results from the XAD-8 fractionation of the original water with that of the three treatments show that the processed water have a significantly lower HPO/HPI ratio. This implies that the Chitosan mainly removes the HPO fraction.

	OM ppm ± 0.2 ppm	Removed by the treatment (weight%)	sUVa A_{254}/TOC (L/cm·mg)	SAR A_{254}/A_{400}	HiX	XAD-8 fractionation, day 0		
						%HPI	%HPOA	%HPON
Raw water	4.3	-	0.061	4.3	0.45	42	34	24
Treatment 1	3.7	14	0.042	6.9	0.42	46	36	18
Treatment 2	2.6	39	0.021	16.4	0.39	51	46	2
Treatment 3	2.0	52	0.021	11.3	0.37	59	39	2

Table 1: Summary of the results of TOC analyses, spectroscopy, humification index (HiX) and XAD-8 fractionation for the raw water sample, treatment 1 (Chitosan 1.75 mg/l), treatment 2 (Chitosan 5.0 mg/l) and treatment 3 (Chitosan 2.0mg/L plus iron chlorid/sulphate 2.0 mg/L).

Optical characteristics

The spectroscopic measurements support the interpretation based on the XAD-8 fractionation. The low dosage treatment (Treatment 1) reduced the sUVa to about 2/3 of the "raw" water. In the optimum dosage treatment (Treatment 2) and the combined treatment (Treatment 3) the sUVa were reduced to 1/3 of the original sample. A decrease in sUVa suggests a greater loss

of absorbing molecular species (i.e. conjunct double bonds), relative to less absorbing (inherently smaller, less aromatic and more hydrophilic) species. This tendency is clear throughout the whole 250-600 nm spectra (Figure 1). No significant difference was found between the spectra of the high dosage treatment (Treatment 2) and the combined treatment (Treatment 3).

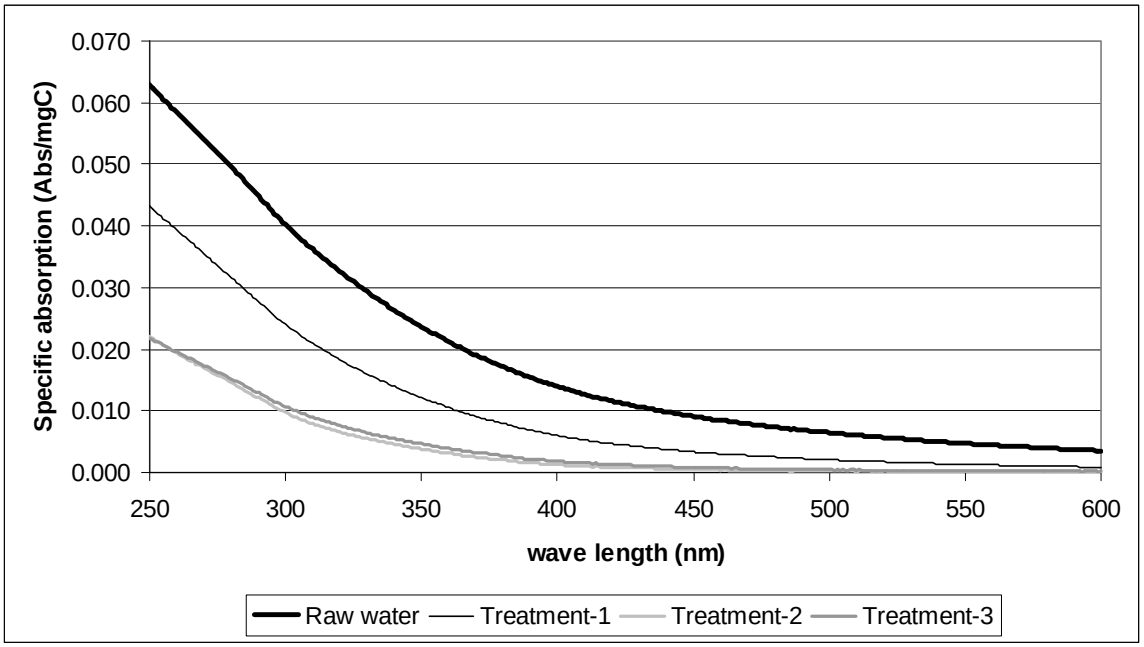

Figure 1: Specific absorption spectra (A_λ/mg C/L) from 250 to 600 nm shows a decrease in specific absorption spectra of the treated samples.

SAR displays an increase with treatment. This loss of colour, or *blue shift* in the spectra, can be interpreted as a loss of longer structures of conjugated double bonds. The increase in SAR is therefore interpreted as a decrease in the relative proportion of more hydrophobic and larger structures of the NOM with the treatment. The results of the "SAR_{300}-spectra" (Figure 2) were in agreement with the classic SAR interpretation. SAR of the combined treatment (Treatment 3) is somewhat *lower* than for the optimum dosage treatment (Treatment 2). Hence, according to the denotation of the SAR this would indicate a relative *higher* proportion of hydrophobic matter in water from the combined treatment compared to the optimum dosage treatment. This result is not supported by the result of the XAD-8 fractionation, however these two analytical techniques are only to be regarded as indicators of size and hydrophobic character and the deviations between the two samples are of minor scale relative to the total change.

The combined treatment (Treatment 3) shows the largest effect in removal of fluorescent compounds from the water. The total decrease in fluorescence is more or less proportional to the total removal of DOC by the three different treatments. Relative fluorescence spectra (Figure 3) show only minor changes with the treatment. The humification index (HiX, Table 1) decreased with increased amount of coagulants in the different treatments. This result further supports that the observed changes in SAR were due to a preferential precipitation of high-Mw structures in the DNOM.

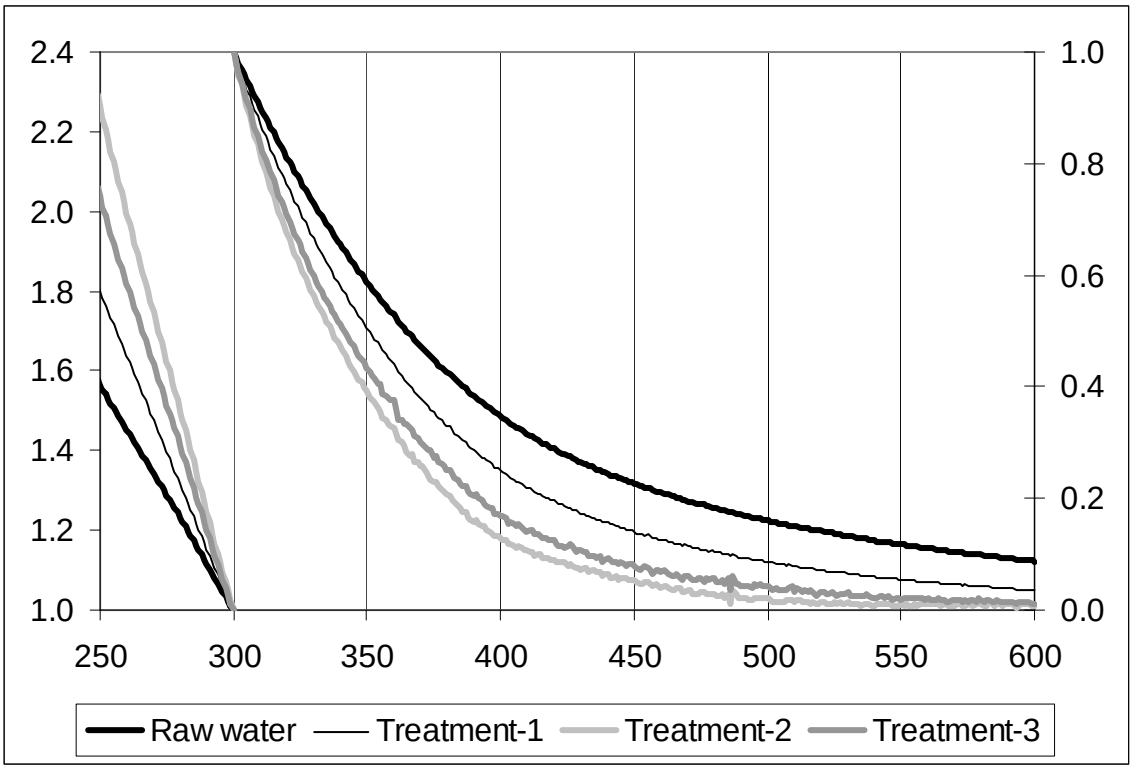

Figure 2: "SAR_{300}-spectra" ($=A_\lambda/A_{300nm}$) from 250 to 600 nm for the raw water and treated samples at day 0 indicate a blue shift in the absorbance spectra of the treated samples. 250-300 nm relates to left y-axis, 300-600 nm relates to right y-axis.

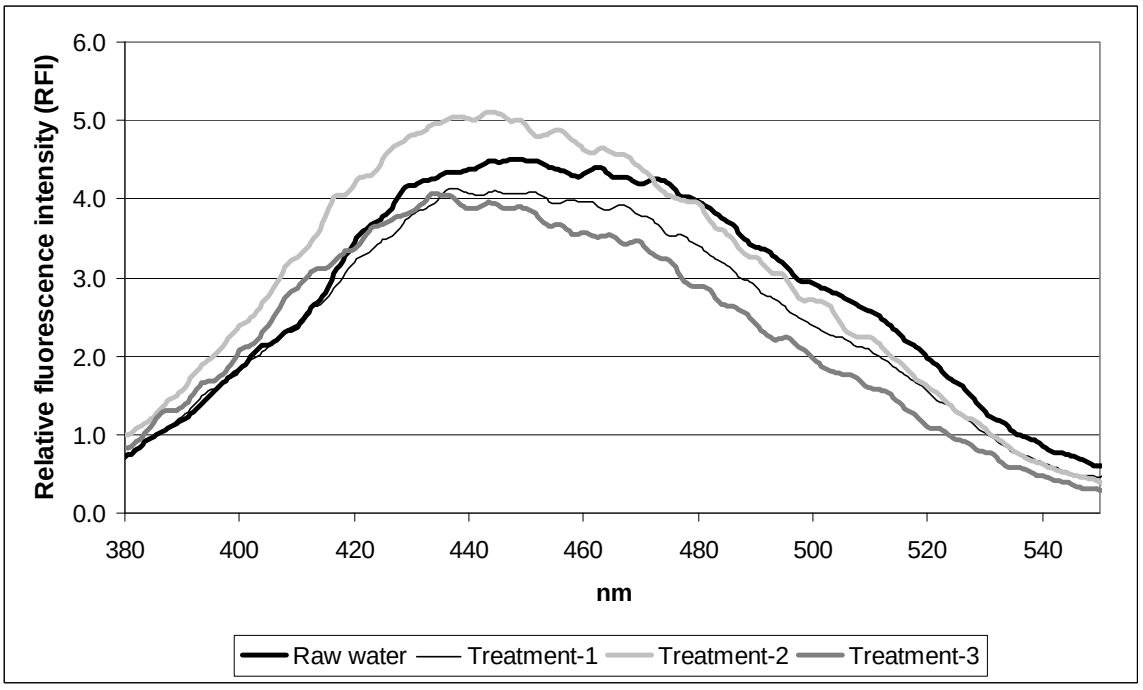

Figure 3: Specific fluorescence spectra (Em/mgC/L) for the raw water and treated samples at day 0 show that only minor changes in the specific fluorescence spectra is introduced by the treatment.

The measured absorption and fluorescence spectra of pure Chitosan solution revealed two major differences compared to spectra of the NOM in the raw water solution. A well defined threshold around 290 nm in the UV-absorption spectra of the acidic Chitosan solution and a maximum of the fluorescence spectrum for Chitosan that occurred at a lower wavelength

(approx. 420 nm) compared to a maximum at 450 nm for the raw water. These differences are however too minor to be distinguished from the spectra of humic matter in solutions with Chitosan in the concentration range used in this study. It is therefore not possible to judge from absorption or fluorescence spectra of treated water whether traces of un-precipitated residuals of Chitosan is present as dissolved OM in the treated water samples.

Comparison of the three treatments

The applied Chitosan dose in the two treatments involving Chitosan alone (Treatment 1 and 2) display a clear linear relationship (i.e. passing through origo) with the total NOM removal. When this relationship is compared with the effect of the combined treatment (3), it can be observed that the combined treatment is more than three times as efficient in total NOM removal than the dose of Chitosan alone in this treatment should account for. 2.0 mg/L iron is a relatively low dose compared with other studies (Vogelsang and Liltved 2001). A synergy effect between Chitosan and iron is a reasonable explanation since the two coagulants operate by somewhat different mechanisms: Coagulation with iron work primarily by charge neutralization and because of its smaller size it has access to a larger proportion of the NOM complexes than the polymer Chitosan. The coagulation of NOM with Chitosan is also to some extent based on charge neutralisation, but the large molecular size of the polymer, inhibit full access to the NOM complexes. On the other hand Chitosan offers the possibility of inter-particle bridging between NOM aggregates and thereby more efficient build up aggregates of a size that would no longer be stable in solution. Since it is of interest to keep the total addition of coagulants to a minimum, these results suggest that the combined treatment of chitosan and iron is the most promising candidate for further studies.

All three characterisation methods (XAD-8 fractionation, absorbance and fluorescence spectroscopy) indicate that it is primarily the larger, more hydrophobic material of highest humification index that is removed by the treatments. No significant differences between the *quality* of the remaining OM of the two most efficient treatments (Treatment 2 and 3) could be observed.

Effect of storage on NOM characteristics

No significant changes were observed in any of the UV-VIS and fluorescence spectra over the period of 28 days. This could indicate that no major changes occurred in the structure of the organic matter during this period and thus that the covalent molecular structure and distribution of conjunct double bonds remain unchanged during the four week storage. However, the XAD-8 fractionations of the same samples revealed major changes in the distribution between hydrophobic and hydrophilic matter already during the 7-days period (Figure 4). The ratio between HPO and HPI, and the relative proportion of the most hydrophobic fraction (HPON) compared with the total sample (HPON/TOT) were used to describe these changes. While no significant changes occurred among the XAD-8 fractions of the raw water sample, major changes in the HPO/HPI-ratio took place in the treated samples. They all displayed a uniform and clear trend of increased hydrophobic character with time in storage, and thereby became more similar to the original raw water sample.

The water quality 28 days after the treatment has in general little practical significance for the original scope of the study since the treated water will only be in the distribution system less than one week. Never the less this XAD-8 analysis would help to verify the fractionation results and place the significant order and rate of the changes in a time perspective. Since little

change took place in the ratio between HPO and HPI matter of the raw water sample, this stability (normal variation) was used as a reference indicator of the statistical significance (measured by t-test) of the changes that occurred in the Chitosan treated samples. On a significance level of 95% it is possible to conclude that for Treatment 3 the changes in HPO/HPI-ratio were significant from day one. Treatment 2 displayed clear significant changes at a 95% level first after 28 days, though the 7th day fractionation already displayed changes at 90% significance. Investigation of the change in the ratio of HPON/TOT gave very similar results, but in this case significant changes were observed first after 28 days. The most remarkable change is observed for the HPON fraction that is almost absent in the samples after Treatment 2 and 3 and that is close to regained in proportion to the relative distribution of the raw water sample after 28 days.

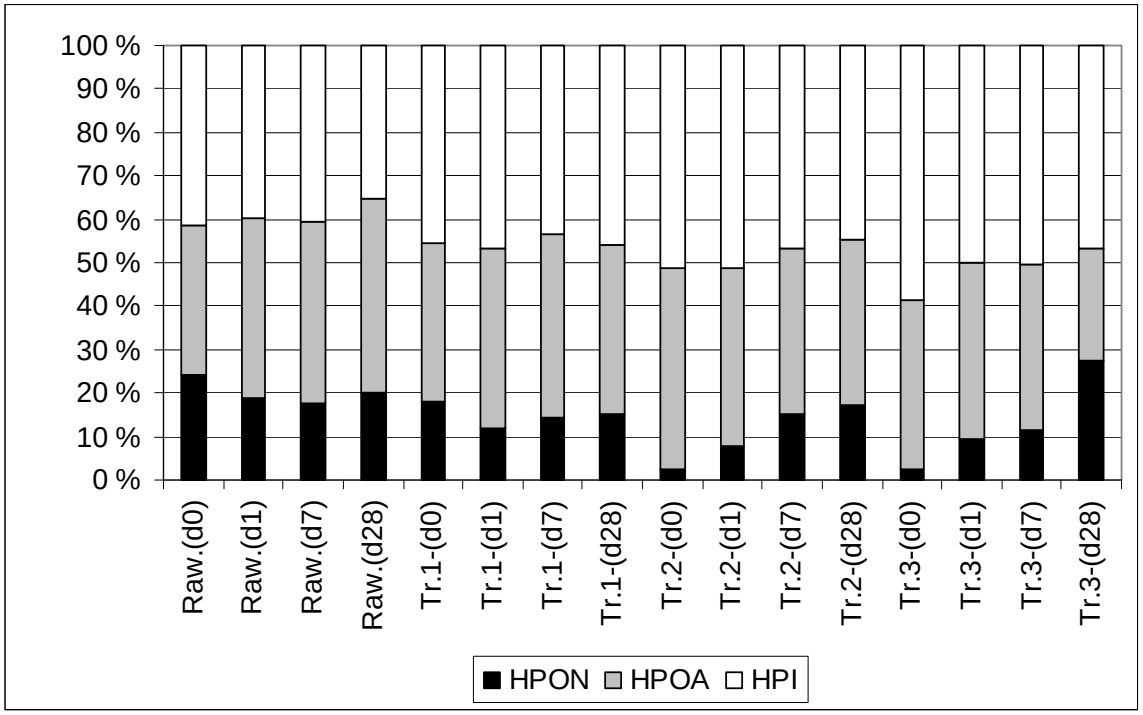

Figure 4: Variation with time in the relative proportions of XAD-8 fractions for the raw water and treated samples indicates that hydrophobic matter (measured by XAD-8 fractionation) is regenerated during storage.

The apparent discrepancy between the storage results on the optical properties and the XAD-8 fractionation could be due to that the changes in the structure of the humic matter does *not* involve changing the frequency and distribution of covalent conjunct double bonds (structure on the micro-scale), but rather the total molecular or aggregate size and charge density of the humic matter. The composition of the NOM organic fractions is likely in some sort of kinetically constrained equilibrium with each other. If one NOM fraction is removed e.g. by preferential precipitation, filtration or chromatographic processes, a "regeneration" of the lost fraction with time by a slow approach towards a new equilibrium between NOM in various degrees of aggregation and colloidal state is therefore likely to occur (Gadmar et al. 2004).

Furthermore, one could conceive that not all of the added flocculants actually precipitate and are removed by filtration, but that a minor fraction remains in the treated sample. This would allow the remaining flocculants to continue to build aggregates after filtration. Such secondary aggregation with Chitosan would provide an increase in aggregate size and an elevated hydrophobic nature (i.e. as measured by XAD-8 fractionation) without leading to major structural changes in the covalent double bond structure of the humic matter. The

practical consequences of such a mechanism of secondary aggregation could be minor or non existent and are beyond the scope of this study.

Conclusions

The coagulants displayed a clear preference toward removing the more hydrophobic matter as measured by both optical properties and XAD-8-frationation. When stored, however, the samples treated with Chitosan or a combination of Chitosan and iron displayed major changes in the ratio between hydrophobic and hydrophilic matter measured by XAD-8 fractionation. During several weeks of storage the hydrophobic/hydrophilic ratio became more and more similar to the original water. No changes were observed in the optical properties of any of the samples. A structural interpretation of the results from the storage test is suggested.

It should be emphasised that we at present are not capable of evaluating the consequence of neither the differences in the quality of the organic matter due to treatment, nor the apparent change of in the HPI/HPO-ratio with time of storage for the response in the distribution system. This will be an important task in the future.

Acknowledgement

This study is performed in cooperation with the Norwegian Institute for Water research (NIVA). Christian Vogelsang from NIVA has performed the preparation and pre-analysis processing of the samples, and has furthermore contributed with valuable information and discussion for which he is gratefully acknowledged. Egil Gjessing and Rolf Vogt from the University of Oslo have also contributed with valuable discussion for which they are gratefully acknowledged.

References

Abbt-Braun G, Frimmel FH. Basic characterization of Norwegian NOM samples – Similarities and differences, Environ Int. 1999; 25: 161-180.

Andersen DO, Alberts JJ, Takács M. Nature of Natural Organic Matter (NOM) in Acidified and Limed Waters, Wat. Res. 2000; 34: 266-278.

Backlund P, Wondergem E, Voogd K, de Jong A. Mutagenic activity and presence of the strong mutagen 3-chloro-4-(dichloromethyl)-5-hydroxy-2-(5H)-furanone (MX) in chlorinated raw and drinking waters in The Netherlands. Sci. Tot. Environ., 1989, 84, 273-282.

Fessenden RJ, Fessenden JS. The basics of Organic Chemistry, Second edition, Allyn and Bacon Inc., Boston, US; 1978.

Gadmar TC, Vogt RD, Evje L. Artefacts in XAD8 NOM fractionation. Accepted. Intern. J. Environ. Anal. Chem. 2004.

Leenheer JA, Huffman EWD. Classification of organic solutes in water by using macroreticular resins, J. Res. US Geol. Surv. 1979; 4: 737-751.

Leenheer JA. Comprehensive Approach to Preparative Isolation and Fractionation of Dissolved Organic Carbon from Natural Waters and Wastewaters, Env. Sci. & Tech. 1981; 15: 578-587.

Malcolm RL. Spectroscopic Approaches. In: Heyes MHB, MacCarty P, Malcolm RL, Swift RS, editors: Humic Substances II. In search of structure. John Wiley & Sons, Chichester, 1989, pp. 303-324.

Miano TM, Piccolo A, Celano G, Senesi N. Infrared and fluorescence spectroscopy of glyphosate-humic acid complexes. Sci. Tot. Environ. 1992, 123/124, 83-92.

Millipore Corporate Headquarters, 290 Concord Rd., Billerica, MA 01821, USA, http://www.millipore.com/ (accessed Oct. 1st 2004).

Moore SG, Calabrese EJ, DiNardi SR, Tuthill RW. Potential health effects of chlorine dioxide as a disinfectant in potable water suppliers, Medical Hypotheses, 1978, 4(5), 481-496.

Rook JJ. Haloforms in drinkingwater. J. Am. Water Works Ass. 1976, 68(3), 168-172.

Senesi N. Molecular and quantitative aspects of the chemistry of fulvic acid and its interactions with metal ions and organic chemicals. Part II - The fluorescence spectroscopy approach. Analytica Chimica Acta. 1990, 232, 77-106.

Senesi N, Miano T, Provenzano M, Brunetti G. Characterization, differentiation, and classification of humic substances by fluorescence spectroscopy. Soil science. 1991, 152(4), 259-271.

Shimadzu. Instruction manual, Total Organic Carbon Analyzer, Model TOC-5000A, Shimadzu Corporation, Tokyo, Japan, 1998.

Thomsen M, Lassen P, Dobel S, Hansen PE, Carlsen L, Mogensen BB. Characterisation of humic materials of different origin: A multivariate approach for quantifying the latent properties of dissolved organic matter, Chemosphere 2002, 49: 1327-1337.

Vogelsang C, Liltved H. ”The use of Chitosan for removal of humic substances at Årnes water treatment plant A/L – the results from jar-tests” (in Norwegian), Norwegian Institute for Water Research (NIVA), Report LNR 4390-2001, Oslo, Norway, 2001.

Vogelsang C, Vårum KM, Mustaperta E, Håkonsen T, Andersen DO, Pedersen MA, Hey A, Müller ED, Jantsch TG. Precipitation of humus by chitosan, Submitted. to Water Sci. & Tech. 2004.

Williams DH, Flemming I. Spectroscopic methods in organic chemistry, Third edition, McGraw Hill Book Company (UK) Limited, London, UK, 1980.

Zsolnay A, Baigar E, Jimenez M, Steinweg B, Saccomandi F. Differentiating with fluorescence spectroscopy the sources of dissolved organic matter in soils subjected to drying. Chemosphere 1999, 38: 45-50.

Paper IV

The effect of forest liming on mobilisation of natural organic matter (NOM) and changes of their physicochemical properties

Tone C. Gadmar[a], Atle Hindar[b], Egil Gjessing[a]

[a] University of Oslo, Dept. of Chemistry, P.O.Box 1033, N-0315 Oslo, Norway
[b] Norwegian Institute for Water Research, Southern Branch, Televeien 3, N-4879 Grimstad, Norway.

Keywords: Terrestrial liming, TOC, mobilization, structure, organic acidity

ABSTRACT

An acidified coniferous catchment was limed with 2.9 t ha^{-1} of coarse-grained dolomite powder in September 1994. Stream-water chemistry in the runoff from the catchment and a corresponding control catchment has been monitored from May 1993 to June 2002. Two RO-isolates were collected from the streams in May 1996 as part of the Norwegian NOM-typing project. A significant increase of about 30% in total organic carbon (TOC) in the stream water was attributed to liming. This study focuses on this increase and change in quality of natural organic matter (NOM). The total concentration of acidic sites was found to increase in the limed NOM material mainly due to an increase in weak acid sites (i.e. with high pKa values). Furthermore the material from the limed catchment was more aromatic and contained a lower content of organic sulphur. Mechanisms for the enhanced TOC mobilization are discussed and related to observed changes in NOM structure and functionality.

INTRODUCTION

Large forested areas of the southern and western parts of Norway have for several decades been affected by acid deposition, resulting in acidification of lakes and streams, and substantial damage to fish populations and aquatic ecosystems (Henriksen et al. 1999, Hesthagen et al. 1999). Liming with calcite or dolomitic limestone has been used to improve the water quality and thereby prevent some of the damage to lakes and rivers. Terrestrial whole-catchment liming has the advantages over the direct aquatic liming of lakes and streams that it attacks the root of the problem; Decreased base saturation (BS) of the soil, caused by the acid rain, along with elevated levels of mobile acid anions enables leaching of H^+ and toxic inorganic aluminium to surface waters (i.e. water acidification). Terrestrial liming increases the BS of the soil allowing the pH of surface waters to increases along with a decrease in inorganic aluminium species. The terrestrial liming will have a more long-term effect and thereby represent a low-maintenance amelioration alternative.

A Norwegian research program “Counteractions against acidification in forest ecosystems” was launched in 1991. As a part of this research program, a whole-catchment liming experiment was conducted in a coniferous forest in Gjerstad in 1994 in the southernmost Norway (Hindar et al. 2003, Hindar 2005). Two comparable catchments were selected, one to be limed with dolomite and one to serve as a control catchment. Water chemistry in the streams draining the two sites was monitored for one year prior to the liming in 1994 and then eight years post liming. The Gjerstad catchments have also been included in the Norwegian “NOM typing project (Gjessing et al. 1999), in which reverse osmosis isolates of natural organic matter (NOM) from eight catchments in Norway were subjected to a large number of chemical and structural analyses.

Major changes in the surface water chemistry after liming was described and discussed by Hindar et al. (2003). Among these is a significant increase in pH, decreased concentrations of inorganic aluminium, along with an increase in the TOC concentration. Several mechanisms could govern this increased leaching of TOC: a) increased terrestrial production, b) increased micro-biological degradation or c) mobilization of less soluble terrestrial NOM. This work explores further the mechanisms behind this elevated mobilization and possible consequences of liming on the NOM structure and properties.

EXPERIMENTAL

Catchments

The Gjerstad experimental site (Hindar et al. 2003) is located in Aust-Agder County in southernmost part of Norway. A paired liming experiment was conducted, where one catchment (84 ha) was selected for liming and a nearby catchment (41 ha) served as a control catchment. Both catchments are forested with mixed coniferous forests: 12-20% broad-leaved trees mixed with coniferous stands. Soils are organic rich acidic podzols. This soil type is commonly found in forests on the nutrient poor siliceous moraine in southern Norway.

Mean annual precipitation is about 1200 mm with a mean annual discharge of 900 mm. Bulk deposition of S and N for the period 1994-1999 was 0.90 g m^{-2} and 1.52 g m^{-2}, respectively. No significant differences in soil chemistry between the two catchments were observed prior to the liming (Hindar et al. 2003).

The limed catchment area was treated with 240 tons of coarse-grained (0-2 mm) dolomite [$CaMg(CO3)_2$] in September 1994, corresponding to a dose of 2.9 t ha^{-1} (Hindar et al. 2003).

Sampling and sample pre-treatment

Freshwater samples

Samples were collected every two weeks from the two streams from May 1993 to June 2002. All samples were analysed 2-3 days after sampling for major chemical constituents and Al-fractions. Water flow in the two streams was monitored at calibrated 120° V-notch weirs (Hindar et al. 2003). All reported data are volume weighted results.

RO-isolates

Reverse osmosis (RO) isolates used in this study are from the Norwegian "NOM typing project" (Gjessing et al. 1999). In this project TOC was isolated by reverse osmosis of 1500-3000 L of stream water from each of the sampling sites, according to the procedure described in Serkiz and Perdue (1990). The isolation took place on site in May 1996, i.e. 20 months after the lime was applied. The water was first pumped through a pre-filter (cellulose 1 µm) and an inline totalizing flow meter into the reservoir of the RO-unit (PROS/2S, Serkiz and Perdue 1990). From the sample reservoir the water was pumped through an inline cation exchanger replacing other cations with Na^+ to prevent precipitation of insoluble salts, such as $CaCO3$(s) and Ca SO_4 (s). The feed water then passed through a pump that boosted the pressure to about 250 psi and through the RO membranes. The pores of these membranes are claimed to be about 150 Å. In the RO membranes the feed water was separated into a permeate solution containing virtually no solutes (< 0.2 mg C L^{-1}; Cond. 0.17 mS m^{-1}) and a

retentate solution that contained nearly all the dissolved inorganic and organic material in the sample. The permeate solution was discharged and the retentate solution was recycled back to the sample reservoir. Additional feed water was pumped into the sample reservoir at a rate that maintained a more or less constant volume (i.e. 25 L) of the reservoir. The capacity of this equipment is about 200 L $hour^{-1}$. These TOC or NOM concentrates were filtered through 0.45 μm filter (Nuclepore) upon arrival to the laboratory. In the laboratory each of the samples was further concentrated by a rotary evaporator (max. 30°C) to a volume of about 5 L and then finally freeze-dried.

Methods

Major chemical analysis of the stream water runoff

The analysis was performed according to standard methods (Norwegian Standard (NS) and European Standard (EN)-ISO (1997)) using inductive coupled plasma (ICP) spectroscopy for major cations and ion chromatography (IC) for anions. Nitrogen species were measured by automatic colorimetry (NS-4743, NS-4746). Total organic carbon (TOC) in stream-water was measured after wet chemical oxidation by IR-detection. Alkalinity is determined by potentiometric titration (Henriksen 1982). Aluminium species were analysed using the pyro-catechol violet method (Røgeberg & Henriksen 1985). Inorganic monomeric aluminium (Al_i) was defined as the difference between the total reactive aluminium (Al_r) and organic monomeric aluminium (Al_o). Organic nitrogen (Org-N) is calculated as the difference between total nitrogen (Tot-N) and the sum of NO_3-N and NH_4-N. Charge balance was calculated and the discrepancy between inorganic cations and anions was assigned negative organic charge of the TOC.

Chemical and structural analysis of the RO-isolates

Determinations of total C, H, and N content of the RO-isolates (Gjessing et al. 1999) were conducted using a Carlo Erba 1106 element analyzer. The content of ash was measured gravimetrically after igniting the samples for two hours at 550° C (Krogstad, 1992). Organic content of oxygen was obtained by subtracting the other constituents from the organic content. Organic sulphur was determined in water-dissolved isolates, as the SO_4^{2-}-difference before and after mineralization of the organic matter by UV/H_2O_2 treatment (1500 W Hg-lamp, 7 cm distance). Sulphate was determined by ion chromatography (NS-EN ISO 10304-1).

Optical properties of water dissolved isolates were measured as absorption scans from 250 to 600 nm on a Hitachi U2000 Spectrophotometer using a 1 cm quarts cell. All samples were measured at the natural pH of the dissolved RO-isolates (pH 6 ± 0.3) and when acidified to pH 2.0 (± 0.1) with 37 % HCl just prior to measurements. Specific Absorption Ratio (SAR= A_{254nm}/ A_{400nm}), specific UV absorption (sUVa = A_{254nm}/DOC) and relative colour (RC = A_{410nm}/DOC) were used as indicators of structural differences between the two samples.

DOC concentrations in the dissolved isolate solutions were determined on a Shimadzu TOC 5000A complying with ISO 8245 where organic carbon (OC) is combusted to CO_2 by means of high temperature and catalysis. The CO_2 is subsequently measured using an IR detector. Combustion temperature was 680°C and platinum was used as catalyst.

For octanol solubility the RO-isolates were dissolved in carbon free water to an approximate concentration of 5 mgC L^{-1} (10 mg organic matter per L, ash content accounted for). Aliquots of these solutions were pH adjusted with HCl to pH 1, 2 and 3. Octanol was added (5 mL octanol to 15 mL sample) and this mixture was carefully shaken for 150 min. The distribution of TOC in the water phase was determined by measuring the absorption at 254 nm.

Liquid state ^{13}C NMR and ^{1}H NMR spectres were recorded by Pempkowiak (2005) using a Varian Plus 500 NMR spectrometer at 125 MHz (0.2 mol/l NaOD in D_2O). The ^{13}C NMR spectra were recorded in solutions containing 100 g L^{-1} of RO-isolate. The probe temperature was 20 °C. The acquisition conditions were as follows: Acquisition time 0.3s; relaxation delay 5.0s; spectral window width 57544 Hz; number of scans 42000. Proton decoupling mode was utilized. The ^{1}H NMR spectra was recorded under the same instrumental and analytical conditions except that the sample contained 20 g L^{-1} RO-isolate and that the number of scans was 5000.

Proton binding capacity of the RO-isolates were analysed and modelled by Takács et al. (1999). The RO-isolate were dissolved to original sample concentration in 0.05 M LiOH to avoid alterations of the concentration of major cations, and shaken under nitrogen for 24 h. The solutions were filtered through 0.2 µm glass fibre filters and adjusted to pH 8 with HNO_3. Ultra filtration was carried out on the DNOM sample solutions through 1000 Dalton molecular weight filter. Each sample was filtered twice and an aliquot of the >1000 Dalton retentate was used for the titration. The samples (15.00 mL) were diluted with 50.00 mL 0.05 M NaCl and acidified to pH 3.5 with 0.1 M HCl and purged with nitrogen. Equilibrium titrations were made twice with 0.05 M NaOH to pH 11 with fixed increments (0.1 mL). Equilibrium back titrations were made with 0.1 M HCl to pH 3.5 with fixed increments (0.05 mL). Interpolation of the fixed titration increment profiles was performed on a fixed titration increment grid (Δ pH 0.5). The pK_a values were determined with Linear Programming with 0.1 pK_a grid, using the pH intervals 4.0-4.9, 6.5-7.4 and 9.0-9.9.

RESULTS AND DISCUSSION

The TOC quantity, free charge and mobilization

The TOC concentration of the streams at the two Gjerstad sites (limed and reference) are closely correlated with the precipitation intensity, displaying high TOC levels at high precipitation and low TOC during dry periods. This is a pattern of TOC discharge that is typically observed in catchments with thin soil, since more of the discharged water during the wet periods flow only through the more organic rich top soils (i.e. sub-lateral flow path). During dry periods the water will mainly drain through deeper and more mineral-rich soil layers.

Prior to liming a lower TOC concentration (about 30%) was measured in stream water draining the limed catchment relative to the runoff from the control site. During the eight years following the liming the TOC concentration in the runoff from the limed site increased significantly relative to the control site (Figure 1) leading to similar TOC levels in the streams in the autumn of 2002. The increase in average TOC concentration in the runoff from the limed site equals about 0.25 mg C L^{-1} y^{-1}, corresponding to 2 mg L^{-1} over the whole period. This increase is significant with a confidence level of 95% when the year prior to the liming is compared with the last year of observation (2001-2002) using the students t-test. A slight increase in TOC is also observed at the control site during the same period, but this increase is

not significant. A general increase in surface water TOC and especially colour has been observed in northern Scandinavia during the last decades (Nordtest, 2003). Still accounting for this general increase in background colour, at least 2/3 of the increase in TOC observed in the limed Gjerstad site can be assigned to the liming.

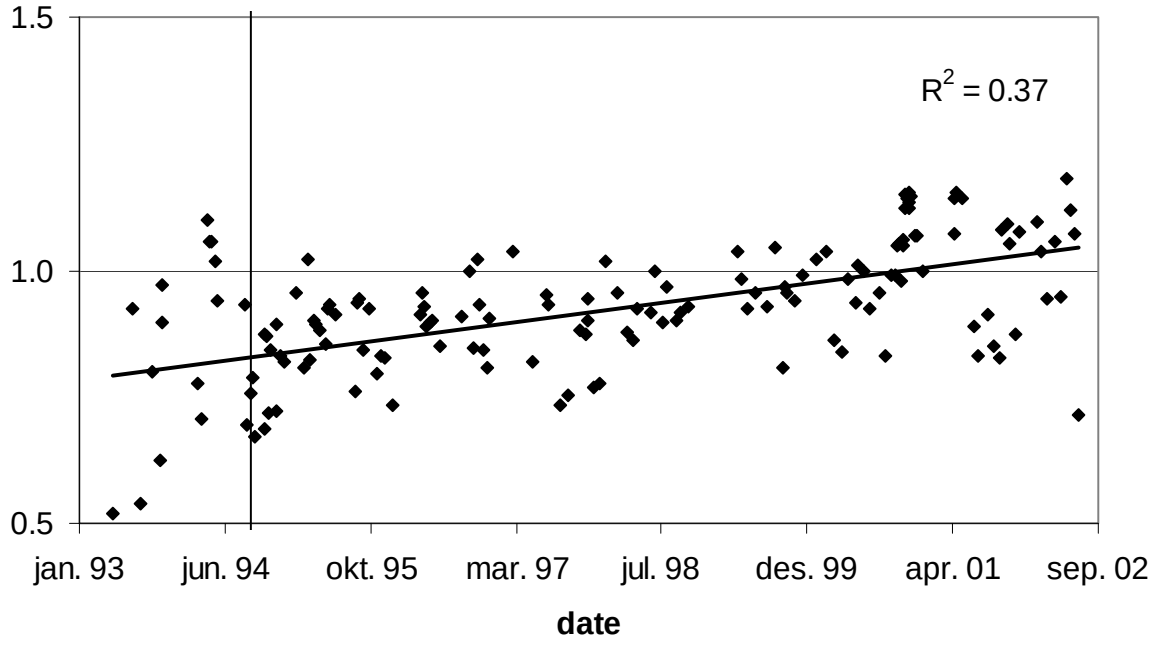

Figure 1: The increase in TOC concentration (mgC L^{-1}) on the limed site relative to the reference site ([TOC] $_{limed}$ / [TOC] $_{reference}$). One outlier excluded. The liming in September 1994 is represented by a vertical line.

As mentioned in the introduction several factors could explain an increase in TOC following as an effect of the terrestrial liming. No change in forest production was observed during the observation period following the liming (Hindar et al. 2003). Thus the hypothesis of increased TOC due to increase in terrestrial production is rejected as this effect probably would need a longer time span and can not explain the rapid change in TOC chemistry observed in the Gjerstad catchment. A second and faster mechanism to explain the increase in TOC could be increased micro-biological degradation of organic matter due to higher pH in the soil. Such an increase in micro-biological turnover of organic matter has been reported in the literature as a possible consequence of liming and increased pH (Mathur and Farnham 1985). It is difficult to confirm this hypothesis directly from the runoff chemistry without measuring an increased presence of soil enzymatic activity, but it would be natural to look for indicators of freshness in the TOC material like a decrease in the C/N ratio, and smaller and more aliphatic compounds.

As a third possibility that could explain increased TOC in the runoff water from the catchment would be increased mobility of TOC already present in the soil matrix. TOC solubility and thus mobilization is dependent on its charge density (the ratio of net charge to carbon). This charge density is dependent on the pH. The pH in the surface water of the experimental site changed from the initial level of 4.5-5.0 to a stable level around pH 6 after liming. Since a fraction of the weak organic acids of the NOM material have pK_a values in this range, a higher level of free charge per mgC is expected. This could be an important factor governing the elevated leaching of the TOC material from the limed site. Free organic charge (FC) has been modelled by subtracting an estimate of the bicarbonate from the acid charge balance and

divided by TOC concentration (Equation 1). An estimate of the bicarbonate concentration was calculated from the alkalinity of the samples. The alkalinity was adjusted for the amount of H^+ consumed by the auto-protolysis of water. This model provides a relatively conservative estimate that is likely to underestimate the average organic charge slightly since some organic acids are protonized during the alkalinity titration.

Equation 1: Free charge per mgC modelled from charge balance (μeq mgC^{-1})

$$FC_1 = \frac{[H^+]+[K^+]+[Na^+]+[NH_4^+]+2[Ca^{2+}]+2[Mg^{2+}]-[Cl^-]-[NO_3^-]-2[SO_4^{2-}]-[HCO_3^-]}{[TOC]}$$

The model displays a clear increase (approximately 25-30% μeq mgC^{-1}.) in average organic charge after the liming of the experimental catchment. This increase in free charge is so large that it alone probably could explain the mobilization of the TOC observed in the treated catchment.

TOC quality of the RO-isolates

The two RO-isolates from the streams draining the limed and control catchments, sampled two years after the liming, show some noteworthy differences. These dissimilarities are in the following explained based on the hypothesis of increased mobilization due to increase in the charge density of TOC.

The proton binding capacity measured by titrations of the RO-isolate from the limed site was significantly higher compared to the control site (Table 1). At the same time the number of acidic sites with high average pK_a-values, increase significantly. When looking at the lowest pK_a interval (4.0-4.9) the increase in binding capacity is only about 20% compared to the reference side. At the second pK_a interval (6.5-7.4) the limed side FC is 50% higher, and at the highest pK_a interval the capacity of the RO-isolate from the limed catchment is more than twice the capacity found at the reference catchment. This remarkable difference in property fits well with the suggested liming-induced mobilization; TOC with a high content of medium to high pK_a valued acids would be mobilized as they are de-protonized by the increasing pH, while the effect on acids with the lowest pK_a values would only be of minor importance since these acids already are active at pH 4.5.

Table 1: The proton binding capacity modelled for three pK_a ranges (Takács et al. 1999).

	RO-isolate from control site Binding site (μeq mgC^{-1})	RO-isolate from limed site Binding sites (μeq mgC^{-1})
pK_{a1} (4.0-4.9)	7.14	8.62
pK_{a2} (6.5-7.4)	4.86	7.49
pK_{a3} (9.0-9.9)	8.23	17.82

Other differences between the two RO-isolates are increased content of aromatic compounds (expressed as a increase in sUVa, ratio of aromatic to aliphatic carbon (ArC/AlC) measured by ^{13}C NMR as well as a slightly higher SAR), a 2 % increase in relative colour (RC), and increased octanol/water partition coefficient (K_{ow}) of the RO-isolate from the limed site compared to the control site (Table 2). Increased aromaticity is in correspondence with the hypothesis of increased mobilization due to higher charge density since the otherwise lesser soluble aromatic TOC could gain increased solubility at higher pH. The most significant difference is found for the bioavailability measured by the octanol/water partition coefficient.

The K_{ow} of the RO-isolate from the limed site is about 30 times higher than from the control site. This could be explained by the higher aromatic content together with the higher average pK_a values of the RO-isolate from the limed site.

Table 2: Optical properties and bioavailability of the RO-isolates (Gjessing et al., 1999)

	Reference RO-isolate	Limed RO-isolate
sUVa (A_{254nm}·L mgC^{-1})	0.042	0.046
RC (mgPt mgC^{-1})	7.9	8.1
SAR (A_{254nm} A_{400nm}^{-1})	7.9	8.4
K_{ow}	0.09	3.06
ArC AlC^{-1}	0.28	0.35

Generally there were only minor differences in the elemental content of the two RO-isolates (Table 3). The relative carbon content is slightly higher in the RO-isolate originating from the limed site, which corresponds well with a higher degree of aromatic structures. The most remarkable difference is the sulphur content which is only about 1/10 in the sample from the limed site compared with the reference. Without further investigation it would be difficult to conclude if the low content of organic sulphur is a result of the liming, different site characteristics or simply coincidence. The sulphate in the runoff decreased in both sites over the observed period with the sulphate concentration of the limed site been slightly higher over the total period (before and after the liming). The mechanism here might have been desorption of sea-salt derived SO_4^{2-} in the soil (Hindar 2005).

Table 3: Elemental content of the RO-isolates (Gjessing et al. 1999).

	Reference RO-isolate	Limed RO-isolate
Ash content (weight %)	49.6	60.6
Org-C (% of TOC)	50.8	53.3
Org-H (% of TOC)	5.6	6.1
Org-O (% of TOC)	41.8	38.5
Org-N (% of TOC, incl.NO_3)	1.5	2.0
Org-S (µgS/mgC)	6.23	0.81

Organic nitrogen of the surface water

The content of organic nitrogen (Org-N) in the natural surface water samples are obtained by subtracting the NO_3-N and NH_4-N from the total nitrogen (Tot-N). No significant major changes were found for the Tot-N, NO_3-N, Org-N nor the C/N ratio of the TOC material from the limed site compared to the reference site throughout the observation period, while NH_4-N decreased significantly. This is an important finding since other studies have found a pH-related increase in nitrate leaching after liming (Nohrstedt 2002). Increased nitrogen leakage is explained by increased microbiological degradation and thus mineralization of TOC. Nitrogen is released in the form of NH_4-N followed by nitrification to NO_3-N.

The total stream concentration of organic nitrogen increased slightly in the limed catchment compared with the reference catchment. This increase does, however, not keep up with the increase in TOC, which results in a slightly decreasing trend in the relative organic nitrogen content of the NOM in the limed catchment. This could be a result of mobilization of older and more humified material due to increased free charge, rather than a direct result of increased microbial bacterial degradation. With no increase in NO_3-N and a significant decrease in NH_4-N in the stream water, there are no signs of nitrogen leakage as a result of

increased bacterial degradation. This does, however, not exclude that micro biological activity has increased as a result of the liming, since the moderate dose of dolomite applied in the Gjerstad experiment may stimulate a more slow increase in the decomposition of the organic matter, in which a slower release of nitrogen species would have time to be consumed and recycled directly by vegetation and micro organisms.

CONCLUSIONS

TOC was mobilised after whole-catchment forest liming with coarse-grained dolomite at Gjerstad, southernmost Norway. The increase in stream-water TOC can to a large extent be explained by increased mobility due to a clear increase in average organic charge. The mobilized TOC had a significantly higher total concentration of acidic sites, along with a higher content of sites with high pKa values. Indications of a higher fraction of aromatic compounds were also found. The relative content of organic nitrogen decreased slightly after the liming which could indicate that older and more humified material was mobilized.

ACKNOWLEDGEMENT

Monika Takács and Jim Alberts (University of Georgia, USA), Per Kristian Egeberg (Agder Collage, Norway) and Janusz Pempkowiak (Institute of Oceanology, Poland) are gratefully acknowledged for lending out their data originally produced for the NOM-typing project to this study and for their valuable discussions. Rolf D. Vogt from the University of Oslo contributed with valuable discussion for which he is gratefully acknowledged.

REFERENCES

Gjessing, E.T., Egeberg, P.K., and Håkedal, J., 1999. Natural organic matter in drinking water – The ”NOM typing project”, background and basic characteristics of original water samples and NOM isolates. Environ. Int. 25(2/3):145-159.

Henriksen, A. 1982. Alkalinity and acid precipitation research. Vatten 38: 83-85.

Henriksen, A., Fjeld, E., Hesthagen, T., 1999. Critical load exceedance and damage to fish populations. Ambio 28, 583-586.

Hesthagen, T., Sevaldrud, I., Berger, H.M., 1999. Assessment of damage to fish populations in Norwegian lakes due to acidification. Ambio 28, 12-17.

Hindar, A., Wright, R.F., Nilsen, P., Larssen, T., and Høgberget, R., 2003. Effects on stream water chemistry and forest vitality after whole-catchment application of dolomite to a forest ecosystem in southern Norway. For. Ecol. Manage. 180, 509-525.

Nohrstedt, H.-Ö., 2002. Effects of liming and fertilization (N, PK) on chemistry and nitrogen turnover in acidic forest soils in SW Sweden. Water, Air and Soil Pollution 139: 343-354.

ISO (International Standard) 1997. Geneva: Int. Standardization Agency.

Hindar, A. 2005. Whole-catchment application of dolomite to mitigate episodic acidification of streams induced by sea-salt deposition. Sci. Total Environ. 343: 35-49.

Krogstad, Tore, 1992. Methods for soil analysis (In Norwegian). NLH report no. 6. Institutt for Jordfag, Ås-NLH, ISSN 0803-1304. 32s.

Mathur, S.P., and Farnham, R.S., 1985. Geochemistry of Humic Substances in Natural and Cultivated Peatlands, in Humic substances in soil, sediment and water (Eds. Aiken, G.R., McKnight, D.M., Wershaw, R.L., MacCarthy, P.), John Wiley & Sons Inc., ISBN 0-471-88274-7.

Nohrstedt, H.-Ö., 2002. Effects of liming and fertilization (N, PK) on chemistry and nitrogen turnover in acidic forest soils in SW Sweden. Water, Air and Soil Pollution 139: 343-354.

Nordtest, 2003. Increase in colour and amount of organic matter in surface waters. Position paper 009. Available at <http://www.nordicinnovation.net/_img/position_paper_9.pdf>, 11p.

Norwegian Standard, 1997. Water analysis. Catalogue of Norwegian Standards 1997. ISSN 0800-1057. Available from PO Box 7048, Homansbyen, 0306 Oslo, Norway.

Oliver, B.G., Thurman, E.M., and Malcolm, R.L., 1983. The contribution of humic substances to the acidity of coloured natural waters. Geochim. Cosmochim. Acta 47:2031-2035.

Pempkowiak, J., 2005. NMR spectra of the NOM typing project, unpublished data (pers.com.), Institute of Oceanology, Sopot, Poland.

Røgeberg, E., Henriksen, A., 1985. An automatic method for fractionation and determination of aluminium species in fresh-waters. Vatten 41 (1), 48-53

Serkiz, S.M., and Perdue, E.M., 1990. Isolation of dissolved organic matter from Suwannee River using reverse osmosis. Water Res. 24:911-916.

Takács, M., Alberts, J.J. and Egeberg, P.Kr., 1999. Characterization of natural organic matter from eight Norwegian surface waters: Proton and copper binding. Environ. Int. 25(2/3), 315-323.